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GENETICS AND IMMUNOLOGY

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I. INTRODUCTION

In recent years as part of the universal process by which every science overlaps sooner or later the field of every other science, genetics and immunology have been finding many points of mutual interest. Immunology has developed as a predominantly "applied" science with a constant reference to the immediate problems of human disease and its treatment, and it is still largely unassimilated into general biology. In fact it is characteristic of the history of immunology that the present interest of immunologists in genetic matters stems mainly from the discovery of the Rh group of blood cell antigens with their very important practical implications in obstetrics.

Attempts to bring the facts and concepts of immunology into more effective and satisfying contact with the other biological sciences have been more frequent in recent years. Landsteiner's influence has been outstanding, and his ideas as developed by Marrack, Heidelberger, Pauling and others have given a strong bias toward the interpretation of immunological phenomena in almost exclusively chemical terms. This attitude has been of the greatest value in bringing a more accurately quantitative technique into immunological investigation and a more precise terminology to its discussion. Nevertheless it must still be maintained that the phenomena of immunity are biological phenomena and are no more expressible simply in chemical terms than those of growth, bodily repair, or reproduction.

Our object is primarily to describe the immunological techniques which have been applied to genetic investigations and to discuss the significance of immunological findings. In our view it is impossible to do this without some discussion of the nature of antibodies and the mode of their production. Some space is therefore given to an attempt to interpret the basic immunological phenomena in terms of modern biological conceptions.

We may first give a brief account of the fields in which genetics and immunology overlap. These are :—

(1) The differentiation of bodily components in different individuals of the same species (or of related species) by serological methods, and the use of these differences as markers in genetic experiments or

observations. The first and most important example is the division of human individuals into four major " blood groups " in terms of antigens present on the surface of their red blood cells. All the other investigations of red cell antigens in human beings and in other animals and birds are essentially similar in character.

(2) The impossibility in mammals and birds of transplanting tissue from one individual to another has been shown to be due essentially to the development of immunological responses which prevent the graft's survival. A limited degree of genetic experimentation has been carried out in regard to this phenomenon.

(3) Antibody production, the basic phenomenon on which all immunological investigation rests, has been ascribed to the existence and modification of self-replicating units within the antibody-producing cells with the associated possibility of the passage of antibody-producing power from a cell to its descendant cells (Burnet, 1941). If this conception is established it brings the antibody-producing mechanism into line with the growing number of cytoplasmic entities which have demonstrably, or probably, power of self-replication in response to environmental rather than nuclear stimuli. The nature of the relation between genes and these cytoplasmic entities is a matter of the first importance to genetic theory.

(4) There are genetically determined differences between individual human beings which are in part responsible for the presence or absence of allergic disease. Allergic disease may be defined with reasonable exactitude as disease resulting from inappropriate functioning of immunological mechanisms within the body. Here and there, also, one finds evidence of the existence of strains of laboratory animals which have unusually high, or unusually low, efficiency in producing some type of immune response.

Each of these fields will be discussed, some at greater length than others. Both the authors are immunologists with limited knowledge and no actual experience of genetic work. They can attempt no more than to make available to geneticists information and ideas derived from one science that may serve as a stimulus to advance in another.

2. OUTLINE OF IMMUNOLOGICAL METHODS APPLIED IN GENETIC WORK

The following is a brief resumé of current teaching in regard to the basic immunological concepts of antigen, antibody and immunologically altered cell activity.

An *antigen* is a molecular species, either free or incorporated in the substance of an organism, which when introduced parenterally into a suitable mammalian host (in laboratory practice almost always the rabbit) gives rise to a specific change in the host which may be either the development of antibody in the blood or tissue fluids, or the development of some form of specific tissue reactivity.

Antibody as normally observed consists of molecules of serum globulin which differ from other such molecules in combining readily with the corresponding antigen (or with other substances which have sufficient similarity in chemical structure) but not with unrelated substances. The same aspects of the molecular structure of the antigen (antigenic determinants) as are concerned with antibody reactions *in vitro* are concerned with provoking the reaction (often histamine liberation) of sensitised cells.

The normal type of antigen-antibody reaction observed in immunological work is an *aggregation* reaction in the test tube. If a soluble antigen is used, mixture in appropriate concentration with immune serum results in the formation of a visible precipitate. Current theory regards both antigen and antibody molecules as "multivalent," *i.e.* capable of combining simultaneously with more than one of the complementary molecules. The precipitate appears as a result of the development of a "lattice" of reacting antigen and antibody molecules.

Where cellular antigens are used the aggregation reaction takes the form of *agglutination*, the antibody molecules functioning as bridges to hold the cells together. On occasion anti-red cell sera are titrated by hæmolytic methods, fresh guinea-pig serum (complement) being included in the system to induce hæmolysis of those cells to which antibody has been adsorbed. The antibody recognised by means of a precipitation reaction is called a *precipitin*, that recognised by means of an agglutination reaction is known as an *agglutinin*, and when hæmolytic methods are used for titration the antibody is called a *hæmolysin*. By varying the type of experiment the same antibody molecules may cause precipitation, agglutination, or hæmolysis.

If very low concentrations of antigen only are available, complement fixation methods may be used. These depend on the fact that antigen-antibody complexes too small to produce macroscopic precipitation will actively adsorb complement which is thereby rendered unavailable for the indicator reaction (hæmolysis of sensitised red cells).

Sensitisation reactions have been little used in immuno-genetic studies, but brief reference to them will be made in a later section. The commonest method of demonstrating acquired tissue reactivity is to inoculate the antigen intradermally in the sensitised animal. An erythematous reaction with variable degrees of swelling results. The reaction is not observed in normal animals similarly injected. Other methods involve demonstration of the anaphylactic state in guinea-pigs by either (*a*) injecting the antigen intravenously with production of characteristic symptoms and death, or (*b*) by *in vitro* tests on the contractility of gut or uterine preparations from sensitive animals using the Schultz-Dale technique. A special type of immunologically modified tissue response is seen in experiments involving transfer of living cells by grafting from one animal to another (see section 4).

In genetic work it is common to inject suitable antibody-producers,

usually a series of rabbits, with blood cells from a group of individual animals whose genetic interrelations are being studied. Each sample of cells is inoculated in suitable doses over a certain period of time, into two or more rabbits. Serum samples are obtained about two weeks after the last immunising dose. The immune serum will in general be found to agglutinate red cells of the type used for immunisation to high titre, but it will also agglutinate, often to the same titre, cells from related species of subspecific groups. Since genetic studies can only be concerned with differences it is desirable to eliminate from the serum all antibody which is common to the two composite antigens used. The procedure used is derived from the analogous bacteriological technique and consists in *cross absorption.* If two individuals, X and Y, are being examined for serological differences in their red blood cells, anti-X and anti-Y sera are prepared in rabbits. Anti-X serum is absorbed with Y cells and anti-Y serum with X cells. Samples of the absorbed sera are then tested with both X and Y cells; a sharp difference of the type shown in the table will be evident, provided there are real antigenic differences between X and Y types of cell.

TABLE

Cross absorption of anti-red cell sera

Serum	Absorbed with	Titre against	
		X cells	Y cells
Anti-X	—	20,000	10,000
	X	<100	<100
	Y	8,000	<100
Anti-Y	—	20,000	20,000
	X	<100	10,000
	Y	<100	<100

In general the homologous antigen will absorb all antibody out of an antiserum; any heterologous antigen will leave behind at least some of the antibody reacting with the homologous antigen. There are probably two different reasons for this behaviour when the complex antigens such as blood cells are used. In the first place several distinct antigenic molecular species may be involved. One type of cell may be formulated as containing antigens A, B, C and D, the other A, B, E and F; the antisera will contain corresponding antibodies *a*, *b*, *c*, *d* and *a*, *b*, *e*, *f*. Cross absorption will give sera containing antibodies *c*, *d* and *e*, *f* respectively. It has, however, been shown in several instances that the population of antibody molecules which is produced by a single antigen A is not uniform, but contains a proportion of antibody which can be differentiated by its behaviour on absorption with related antigens A′ A″, etc. (Landsteiner and

van der Scheer, 1936 ; Burnet, 1934). This second type of difference between antigens is particularly to be suspected when close quantitative control of absorbing doses is necessary to obtain specific serological reagents.

The degree of specificity of an antibody response varies with circumstances. There are few data on the point available from work with purified antigens but much from studies with pathogenic micro-organisms. Comparison of antibody reactions to immunisation with strains of influenza virus A have been made in horses, ferrets, and rabbits, and in human adults and children. Horse antisera show little specificity, neutralising to approximately the same titre strains that are widely separate when tested with ferret or rabbit immune sera. Rabbit sera in their turn detect differences between strains identical by ferret antiserum tests. Adult human antisera show a much broader (less specific) response than those from children under twelve.

In dealing with antibody to body constituents, notably red cells, the highest degree of specificity is obtained by *iso-immunisation*, *i.e.* immunisation of another individual of the same species. This method has been widely used in studies of fowl (Todd, 1930) and of bovine red blood cells (Ferguson, Stormont, and Irwin, 1942). Occurring under natural or accidental conditions in man it may give rise to a whole series of important clinical phenomena.

3. EXPERIMENTAL AND CLINICAL DATA IN REGARD TO ANTIGENIC DIFFERENCES IN THE CELLS OF WARM BLOODED VERTEBRATES

For reasons that have already been suggested, work in the field of antigenic differences between individuals has been largely dominated by the importance of such differences in regard to blood transfusion in human beings. A second stimulus to experimental work has been derived from attempts to replace a diseased organ by the same organ or tissue from a healthy donor and from studies of the limitations of transfer of malignant tumours from one host to another. The bulk of the work which will require review is concerned with these two related types of study.

It must be recognised that the available factual material is completely inadequate for any comprehensive discussion of the gene-antigen relationship. Practically all the material concerns " expendable " cells of mammals or birds, which have an elaborate system for disposing of worn-out individual cells and for dealing immunologically with foreign substances or micro-organisms. This introduces important complications which will be discussed later. What is badly lacking is any comprehensive data on antigenic variation in the protein or polysaccharide components of some simple organism with a sexual mode of reproduction such as *Neurospora* or *Saccharomyces*. There is no suggestion that such organisms can produce anything equivalent to an immunological response and the inter-

pretation of results would be free of the necessity for considering the " marker " hypothesis to be dealt with later.

In the following sections an attempt has been made to collect the more important available material on gene-antigen relationships.

(a) The human blood groups

There is a much more extensive literature on the human blood groups than on any other genetically determined aspect of immunology and any theory of gene-antigen-antibody relationship is almost driven to this human material to find tests for its validity or otherwise. The following is a brief recapitulation of the position. Blood grouping is concerned with the recognition of certain antigenic determinants (chemical patterns) on the surface of red blood cells by the use of specifically reactive sera which may be obtained from normal individuals, from patients who have reacted abnormally to pregnancy or transfusion, or from animals that have been specifically immunised for the purpose. In all such tests it is found that red cells from certain individuals are agglutinated by some reagent in the serum while cells from others are not. It can be shown that the serum reagent is a globulin with such a configuration that it is bound specifically to a certain molecular pattern on the cell surface. When these globulin molecules have two or more reactive groups, they can serve as a bridge between two appropriate red cells causing their agglutination.

We can first consider what is known of the inheritance of the chemical patterns on the red cells without at present any regard for the significance of the reagents by which they are detected.

The classical blood groups, O, A, B and AB are differentiated by the presence of one or another of four possible patterns on the surface of the red cells of human beings. Two of these patterns, A and B, are straightforwardly detected by the appropriate agglutinin. A_2 is a less reactive form of A, only to be detected by a proportion of the sera which react with A_1 while O was originally defined on purely negative characteristics. It is now known that O cells have a corresponding pattern (also present to some extent in A_2 and possibly other cells) which can be detected by appropriately chosen animal sera. Omitting very rare anomalies all human bloods fall into one of six patterns :—

O A_1 A_2 B A_1B A_2B

The inheritance is usually interpreted as being dependent on four parallel allelomorphs *O*, A_1, A_2, *B* of which A_1, A_2, and *B* are dominant to *O* and A_1 dominant to A_2. The constitution of the different phenotypes then is :—

Phenotype	O	A_1	A_2	B	A_1B	A_2B
Genotype	*O O*	A_1O	A_2O	*B B*	A_1B	A_2B
		A_1A_2	A_2A_2	*B O*		
		A_1A_1				

Quite early in the history of blood group investigation it was found that a proportion of group A individuals provided secretions (saliva, urine, or gastric fluid) containing the same antigen and therefore capable of "blocking" agglutination of their red cells by an anti-A serum. It is now known that the blood group substances in these secretions are associated with mucins or mucoids and serologically active mucoids of types A, B, and O have been prepared. The most suitable source is the content of pseudomucinous cysts of the ovary. All these mucoids are of very similar chemical character no difference being detectable in the polysaccharide "nucleus" (Morgan, 1947). Individuals can be divided into *secretors* and *non-secretors* according to whether the appropriate blood group substance is present or not in water-soluble form in their secretions. The difference is inherited in simple Mendelian fashion, secretor being dominant to non-secretor.

Serologically active mucins are of wide occurrence amongst the mammalia. Aminoff, Morgan, and Watkins (1946) have recently shown that individual pigs will provide either A or O type mucin from their gastric mucosæ. Monkeys of the genus *Macaca* have red cells which are unagglutinated by human α (anti-A) or β (anti-B) isoagglutinins, but their secretions contain A, B, or O substance and their sera have α, β, or no agglutinins, according to the same rules as human beings. This seems to indicate that the division of human blood cells into the A B O groups may be only a minor consequence of some deep-seated attribute of mammalian organisation.

With the development of methods for detecting O antigen, facts have been uncovered which require some modification of the classical theory of the A B O blood groups. In the first place secretors of blood group A or B who secrete A and B substances in a water-soluble form also secrete O-substance (Morgan, 1947). Secondly, Hirszfeld and Amzel (1940) claim to have demonstrated O-substance in the red cells of individuals "homozygous" for A or B by quantitative agglutination tests with natural bovine anti-O sera, and with high titre anti-O sera produced by the prolonged immunisation of goats with Shiga dysentery bacilli.

To explain these findings Hirszfeld (1947) has postulated the existence of "pleiades" of blood cell types, arbitrarily designated O.... A_5 A_4 A_3 A_2 A_m A_r A_j.... A_c and O.... B_2 B_m B_r B_j.... B_c in decreasing order of reactivity with anti-O serum. These pleiades represent successive mutations from the original primitive group O towards the limiting cases of types A_c and B_c, which contain no O-substance in the cell, but do contain anti-O isoagglutinins in the serum. Individual genes close together in the transition series show the phenomenon of partial dominance whereas those farther apart show absolute dominance. Thus red cells of individuals of the homozygous genotype A_j A_j and of the heterozygous genotype A_j O react equally well with anti-O serum because the gene A_j which

confers a weak but definite O character to the cells, suppresses more or less completely the effect of any genes tending to confer stronger O character. This concept of gene structure will satisfactorily explain the occurrence of O substance in most human red cells, but Hirszfeld's results should perhaps be confirmed with either a human anti-O serum, or a less completely heterologous serum, such as for instance, the anti-O serum produced in rabbits by Morgan and Waddell (1945).

The MN series of blood groups is determined by the use of sera from rabbits which have been immunised with human cells. The sera are then absorbed with heterologous human cells to remove all but the required M or N agglutinins. With such reagents cells can be divided into three groups according as they are agglutinated by M, N, or both sera. No cells are found which are inagglutinable by either serum. The inheritance is independent of sex or of any other type of blood group and is of the simplest Mendelian character.

Phenotype . . .	M	N	MN
Genotype . . .	*MM*	*NN*	*MN*

Certain pig sera agglutinate about two-thirds of a random collection of human blood cells. The antigen P is distinct from any of the other blood groups and is inherited as a dominant Mendelian character.

Phenotype . . .	P+	P−
Genotype . . .	*Pp*	*pp*
	PP	

Recent work (Snyder, 1946) indicates that minor differences in N antigens and in P antigens essentially similar to the A_1—A_2 relationship can be detected by refined methods.

Finally the complex and clinically important group of Rh antigens must be considered. A significant new antigen in human cells was first demonstrated by Levine and Stetson (1939) as a result of a severe transfusion reaction in which donor cells of the same group (O) as the patient had been used. The recipient was shown to have in her serum an agglutinin active against about 85 per cent. of human group O cells. Soon afterwards it was shown that the antibody produced by immunising rabbits or guinea-pigs with *Rhesus* monkey blood agglutinated the same 85 per cent. of human cells. These were therefore described as Rh+ cells the other 15 per cent. of individuals having Rh− cells. Clinically, sera having this type of activity were observed after repeated blood transfusions, some at least of the donors being Rh+ and the recipient Rh−, or in women, themselves Rh−, who had borne more than one Rh+ child. In the latter cases the Rh+ infants were in a proportion of instances subject to more or less severe symptoms of blood destruction shortly after birth.

The inheritance of the two blood cell types was first ascribed to

the existence of two allelomorphs Rh and rh, Rh being dominant to rh. This simple statement is sufficient to cover most of the occurrences, but more detailed study gives a much more complex picture. Fisher's interpretation (Race, 1944, 1946) is that the inheritance is determined by genes situated at three closely adjacent loci on a single chromosome, each gene having two common allelomorphs and possibly other rare ones. The genes are *C*, *c* (C^w); *D*, *d* (D^w); *E*, *e*. In England the three commonest combinations are *CDe* (R_1), 46 per cent.; *cde* (r), 38 per cent.; *cDE* (R_2), 12·8 per cent. Typical Rh positive genotypes are *CDe/CDe*, *CDe/cde*, *cDE/CDe*, while the Rh negative formula is *cde/cde*, or any other combination containing *..d../..d..*

Each allelomorph corresponds to and determines the formation of an antigen on the surface of the red cell, and each antigen may, on introduction into an individual lacking it in his own cells, provoke the appearance of the corresponding antibody. There seems to be no doubt that the D antigen is intrinsically more likely to produce antibody than any other of the antigens, and the standard Rh antiserum which agglutinates 85 per cent. of cells from a normal European population is anti-D.

The details of the process by which a fœtus of genetic composition, *CDe/cde*, can stimulate its *cde/cde* mother to produce anti-D with subsequent passage of the anti-D to the fœtal circulation are still far from clear. The number of cases of damage to the fœtus is far smaller than the number of pregnancies potentially capable of producing it. A repetition of the antigenic stimulus, *e.g.* a second or subsequent pregnancy of the same type, increases the likelihood of antibody production. Individuals apparently differ in their capacity to respond to a potential antigenic stimulus despite the fact that they are nominally of the same Rh genetic constitution. In an experimental study of the artificial immunisation of Rh negative (*cde/cde*) volunteer donors with Rh positive (*cDE/cDE*) cells, Wiener and Sonn-Gordon (1947) obtained only two satisfactory sera from nine subjects. When immunisation was prolonged, anti-D was first produced, followed by anti-E antibody.

The immunising potency of the antigens corresponding to the various Rh genes varies considerably. Of those corresponding to the dominant genes C, D, and E, all will provoke antibody formation whether present in the homozygous or heterozygous condition; but D is a more effective antigen than C, which is in turn better than E. The effect of the antigens corresponding to the recessive genes is inhibited in the heterozygous condition and, even when cells of an individual who is homozygous recessive for one of the genes are used to immunise the corresponding homozygous dominant individual, they are poor antigens. Consequently anti-e, anti-d, and anti-e sera are all very rare.

There is a very extensive recent literature on the subject and as

yet there is no general acceptance of the nomenclature based on Fisher's scheme. Wiener, Sonn, and Polivka (1946) use the older nomenclature and consider that there are six major and perhaps some minor allelic genes on the one chromosome, the "*Hr* factors" (*i.e. cde* in the alternative notation) being related to the *Rh* factors (*CDE*) as *M* is related to *N*.

It appears that the four groups of antigens (ABO, MN, P, and Rh groups) do not exhaust the possibilities of detecting all differences between individuals, but discussion of other differences, mostly based on the reactions obtained with a single serum, is hardly called for in the present context. From all the work that has been covered the main deduction to be made is simply that variation in the nature of a single gene is sharply and regularly reflected in the specific immunological pattern of a red cell surface component. This holds for at least four unrelated series of genes.

From the general point of view, the ABO groups are the most interesting, for they are determined by the use of *natural isoagglutinins*, *i.e.* antibody-like agents not resulting from artificial or accidental immunisation and effective against red cells from certain other individuals of the same species. In man the agglutinin content of any individual serum is determined by the rule that only those agglutinins which do *not* affect the person's own cells are present. A group A person has β agglutinin reacting with B or AB cells, a group O individual both α and β, and so on. This must, however, be qualified in regard to anti-O agglutinins. Sera can be found (amongst natural bovine sera) or prepared (*e.g.* by prolonged immunisation of goats with Shiga dysentery bacilli) which specifically agglutinate human O cells, but natural anti-O isoagglutinin is found only very rarely in human beings (Landsteiner and Witt, 1926).

Three explanations have been put forward to account for the occurrence of the natural human isoagglutinins.

(1) It is characteristic of the globulin molecules produced by the human body, once it is adapted to extra-uterine existence, that a proportion of them can be specifically adsorbed to A and B cell substances, and will therefore act as agglutinins. Essentially this is an accidental occurrence as fortuitous as the fact that certain ox sera will agglutinate human O cells, a mere matter of the chance complementary arrangement of globulin and polysaccharide patterns. When genetic conditions produce A substance in the cells, secretions, and serum of an individual, this will react with the α agglutinin fraction of serum globulin "absorbing" it out of the serum and leaving only the β component. In similar fashion B substance will remove β leaving α, while in an O individual both α and β can remain.

The difficulty of this theory of Bernstein is that it tacitly assumes in A, B, and AB individuals a continuing production of agglutinin which is steadily taken up and removed from serum by cells, or by other sources of blood group substance. It would therefore be expected

that the cells would be prone to spontaneous agglutination or at least give evidence of globulin coating by Coombs test (Coombs, Mourant, and Race, 1945). In fact there is no suggestion of this.

The smallest modification of this hypothesis that might bring it into line with this absence of the evidence for existence of *e.g.* α agglutinin production in an A individual is to assume that the existence of A substance in the agglutinin-producing cells automatically prevents the liberation of the corresponding α agglutinin or, more correctly, of those globulin molecules which have a configuration causing them to act as α agglutinins. As is so often the case in speculative hypotheses in this general field, it must be confessed that on present knowledge it would be almost equally legitimate to assume that the presence of A substance in the cell would stimulate it (in immunological fashion) to produce excessive α agglutinin as to make the present assumption that it will inhibit α agglutinin production.

(2) The α and β agglutinins are essentially similar to other antibodies in being produced in response to an appropriate immunological stimulus. This stimulus is provided at the time of birth, and implants in the antibody producing cells a capacity to produce agglutinin which fades out very slowly after childhood.

The features that favour this hypothesis are :—(1) The disappearance in the first 10 days after birth of the low concentration of iso-agglutinins received across the placenta and the appearance of agglutinin corresponding to the infant's genotype at about 10 days. (2) The existence of a wide range of titres of iso-agglutinin, as of any common acquired antibody. (3) The peak of iso-agglutinin titre in childhood declining slowly thereafter. (4) The fact that the agglutinin titre can be raised by "immunisation" of volunteers with group specific substance, and (5) the correspondence in specificity of α and β iso-agglutinins with those produced in rabbits with semi-synthetic antigens (Morgan and Watkins, 1945).

The major and, at the moment, insuperable difficulty of this hypothesis is to identify the immunological stimulus. To take an infant born of two O parents and therefore O itself, we have to assume that about the time of birth both A and B antigens are introduced into the circulation in sufficient amount to impress a lasting antigenic stimulus on all the cells concerned in this particular type of globulin production. There is no known source of such A and B substances, and until such a source is established the hypothesis will remain untenable.

(3) The last alternative which is supported by Landsteiner (1946) is that the natural agglutinogens and agglutinins must both be accepted as being genetically determined without the introduction of any immunological conceptions whatever. This is an intellectually unsatisfying conclusion and we feel certain that it will eventually be replaced, but at present it is the only available interpretation.

(b) *Red cell antigens in other mammals*

Normal iso-agglutinins can be found in the sera of several other mammals including cattle, sheep, pigs, and horses, but there is not the same complete reciprocal relationship as in human beings. In horses the relationship holds for only two agglutinogens and agglutinins, four other iso-agglutinins occurring in only a small proportion of the animals which lack the corresponding agglutinogen.

Wiener (1943) has summarised the available data on intraspecific variation in red cell antigens in mammals. In general, the antigens are classified by their reactions with antisera prepared either in rabbits, or by iso-immunisation of another individual of the species under study. In either case the presence of a specific antigen accompanies the dominant allele of a specific gene.

It is of interest that an antigen responsible for individual differences in one species may be characteristic of the species as a whole in another. The human blood group A substance is present in all sheep cells while an antigen very similar to B is present in the cells of rabbits, cattle, and cats. The M antigen is found in all rhesus monkeys. This is probably no more than an indication that there is a relatively limited number of molecular patterns which can be developed by the mucins or other components of the cell surface.

Todd and White (1910) showed that there were large numbers of different antigenic types in cattle blood, and this work has been elaborated by Ferguson (1941) and later by Irwin and his colleagues. Owen, Stormont, and Irwin (1947) have shown that there are at least thirty inheritable cellular antigens in cattle, detectable by hæmolysin tests with suitably prepared cow and rabbit sera. Many of the differences between individuals are minor ones detectable only with low dilutions of antiserum. The majority of the antigens appear to be separately inherited, although certain are linked in such a way as to indicate that they are the product of allelic series of genes or of closely linked genes.

An extremely interesting incidental observation from this work on cattle has been provided by Owen (1945) in his discovery of the existence of erythrocyte mosaics in calves from certain multiple births. The production of "freemartins," sterile heifers, in cases where male and female twins develop a common placental circulation, is well known. Under circumstances where twins (or in one instance quintuplets) have a common circulation, two types of red cell may be found in both the twins for life. One corresponds genetically to its own cells, the other to its twin's. This finding seems to have been completely established and has the important implication that cells "foreign" to the host may be tolerated indefinitely provided they are implanted early in embryonic life.

(c) *Red cell antigens in birds*

Todd (1930) obtained evidence of multiple antigenic factors in the blood cells of fowls, these being inherited as simple Mendelian dominants. In most of his work he used polyvalent iso-immune sera, which were appropriately absorbed for use in critical tests. If such a serum was absorbed with cells from both parents, it regularly failed to agglutinate the cells of the offspring although active against all or most unrelated birds. Wiener (1943) considered that Todd's results could be interpreted on the basis of a small number of factors determined by three allelic genes and another separate pair of genes.

Irwin, Cole, and Cumley of the University of Wisconsin have in the last ten years made extensive studies of genetically controlled interspecific variation in pigeons and doves. They used antisera produced in rabbits to investigate the antigens of the erythrocytes of several species of pigeons and doves, of various species hybrids, and of backcross matings of these hybrids with one or both of the parental species.

The findings may be summarised as follows (Irwin and Cumley, 1943 ; Irwin, 1946). For any two species there are in the erythrocytes both common and species-specific antigens which can be detected with appropriate antisera. The antigens observed were all " major " antigens, *i.e.* they were detected with antisera diluted 1/60 and usually 1/200,000 or higher. Thus, in the pearlneck dove (*Streptopelia chinensis*)-ring dove (*S. risoria*) hybrid there may be present antigens ABCD, whereas pearlneck only has ABC and ring dove BCD. A is specific to pearlneck and D to ring dove, B and C being common to each. Backcrosses of the F_1 hybrid to the parental species show that species-specific antigens are inherited as though they were determined by dominant genes. The pearlneck-ring dove hybrid backcrossed to ring dove shows that there are at least ten erythrocyte antigens specific to pearlneck. Since the genes determining these are heterozygous in the F_1 while the ring dove parent is homozygous recessive, the backcross progeny segregate in a one-to-one ratio of presence or absence of each of the ten pearlneck-specific antigens. Backcrossing in the other direction is difficult, since the females, neither of pearlnecks, nor of the species hybrids, produce viable offspring. Extending the same method of immunogenetics, Irwin and Cumley have found similar shared and species-specific erythrocyte antigens in several other species of dove and pigeon.

In all the instances so far described, in humans, cattle, birds, etc., a one-gene-one-antigen relation obtains, although in their earlier papers, Irwin and Cumley (1943) did not postulate such a relationship, but assumed only that each of the ten species-specific antigens of pearlneck, for instance, is produced by one or more genes on as many chromosomes. However, Irwin and Cumley (1945) have described " hybrid substances " in the cells of species-hybrids in doves,

i.e. cellular antigens not present in either parental species. When antisera to pearlneck-ring dove hybrids were absorbed with cells from both parental species, antibodies specific for the hybrid substance complex remained. They explained the phenomenon by assuming that hybrid substance is produced by interaction of genes each of which in the parental species produces only species-specific antigens. We feel that this conception may need modification if and when the antigens concerned are chemically characterised. Irwin and Cumley (1945) have themselves shown that the hybrid substance can be differentiated into two well-defined antigens with a possible third. One is associated with their antigen d 11, the other with any one of several antigens. It seems possible that, although only the parental antigens are induced in the hybrid cells, the new necessities of fitting in the various molecular types into the cell surface mosaic may fortuitously require that new antigenic patterns appear. These would then be determined by stereochemical factors and only in a very indirect way by gene action.

It is not inconceivable that such reorganisation of the cellular surface might even allow the hybrid cells to be antigenic when injected into one or other of the parents. McGibbon (1944) obtained analogous results with duck hybrids, and he was in fact able to produce antibodies against the hybrid substance in the actual parents of the hybrids carrying this new antigen.

(*d*) *Species specificity of proteins and haptens*

The specificity of the cell antigens which we have just been discussing depends upon protein-free polysaccharides and lipids which, when isolated from their usual protein carrier, are highly active *in vitro* but fail to stimulate the production of antibodies. For such substances Landsteiner (1921) proposed the term *hapten*.

There is abundant evidence, which has been summarised by Landsteiner (1946), that proteins are also species-specific. The only systematic investigation of the inheritance of protein specificity is that carried out by Cumley, Irwin, and Cole (1943) on the serum proteins of their dove hybrids. They concluded that species hybrids possessed the proteins of each parent species and that the proteins are controlled by gene action and consequently segregate in backcross hybrids, just as do cellular agglutinogens. The genes responsible for serum antigens appeared to assort independently of those determining the red cell agglutinogens of the same species.

Apart from earlier reports which have not been substantiated (Landsteiner, 1946) only one instance of apparent intra-specific differences in protein has been described. Cumley and Irwin (1942) found individual differences in human sera by means of precipitin tests with absorbed rabbit antisera that may be referable to proteins; but this work needs confirmation.

Landsteiner (1928) has pointed out that there appear to be two systems of species specificity in animals. In the case of proteins the serological and chemical properties in general parallel closely the zoological or bacteriological relationships and the changes are gradual, intra-specific differences being rare or non-existent. On the other hand, the complex antigens consisting of combined protein and hapten are characterised by heterogenetic reactions, which reveal apparent relationships between widely varying species (*e.g.* the Forssman antigens), and by well-defined heritable intra-specific differences, such as those of red blood cells and tissue cells and the type specificity of many bacteria. The relationship between chemical structure and the ease with which mutations can be recognised by changed serological specificity may throw considerable light on the mechanism of evolution. If Tatum and Lederberg's (1947) demonstration of recombination of characters in mixed cultures of the bacterium *Escherichia coli* is confirmed and amplified, bacterial genetics may provide the most direct attack on this problem.

4. TISSUE TRANSPLANTATION

Although the study on one particular form of tissue transplantation, namely the transfusion of red blood cells, has from the first been regarded as a province of immunology, it is only recently that it has been generally accepted that the response of a host to the transplantation of homologous or heterologous normal or tumour tissue is primarily immunological.

In general, if living skin cells are grafted from one site to another on the same individual (*autograft*) they become attached to the raw area, proliferate and eventually reconstitute the normal skin epithelium. If the cells from another unrelated individual of the same species (*homograft*), or of another species (*heterograft*), are used, preliminary attachment occurs, but in 10-14 days there are signs of inflammatory activity beneath the grafts which rapidly separate and necrose.

The most comprehensive account of the results of tissue transplantation is contained in Loeb's (1945) *The Biological Basis of Individuality*. Throughout his work Loeb was very impressed with the lymphocytic infiltration which occurs around all tissue grafts except autografts. He considered that this reaction, and the subsequent destruction of tissue in homo- and hetero-transplantation, was due to the action of " individuality differentials " of the transplant upon the host. He described such differentials as homotoxins and heterotoxins and thought that the effect was a direct toxic action of the graft upon the host, immune reactions being of minor and secondary importance. There is little in Loeb's work, however, which could not be interpreted in terms of the immune response as the principal mechanism, and not a secondary effect. It appears to us that the " individuality differentials " of which he speaks are in fact the antigens of the grafted tissue relative to the particular

host concerned. Loeb and his collaborators carried out experiments involving multiple grafts of thyroid and cartilage ; but they examined the sections only a few days after the second transplantation, and used organs from different donors from the first- and second-set grafts ; it is thus not surprising that they failed to obtain conclusive evidence of the immunological nature of the response to homotransplants.

However, Medawar's studies of homologous skin grafts in humans (Gibson and Medawar, 1943) and rabbits (Medawar, 1944, 1945) conclusively established the immunological nature of the reaction of a host to a homologous tissue graft. He adopted as his criterion of the immunological nature of the reaction the difference in the behaviour of two skin grafts from the same donor implanted on another animal at an interval of 14 days. He found a greatly accelerated reaction to such second set grafts, but not to second grafts from another donor animal. In addition he was able to show that the " dosage " of donor cells, *i.e.* the size of the graft used, influenced the intensity of the response to a second graft from the same donor. The bigger the primary graft the more active the second response. His interpretation is that in response to the primary stimulus an altered state of immunological reactivity is induced which in 12-14 days results in the destruction of the graft. A second graft from the same source finds the reactive state already established and the destructive process begins as soon as the graft cells commence to multiply.

In the field of tumour transplantation the results are not quite so straightforward. Gorer (1938, 1942) insisted that immunity to tumour transplants was identical with immunity to transplants of normal tissue, both being primarily immunological reactions. In studies of sarcoma and leukæmia in mice he produced antibodies in the serum of mice of a resistant strain by the intraperitoneal or subcutaneous inoculation of tumour tissue. When mixed with tumour tissue and inoculated into susceptible mice the antibody exerted a protective effect. However, such experiments as those of Greene (1941 *a* and *b*) on the successful transplantation of a variety of heterologous cancerous tumours to the anterior chamber of the eye of rabbits, rats, and guinea-pigs, when transplants of normal adult heterologous tissue failed, indicated that some additional factor entered into the picture. Greene and Murphy (1945) found that homologous and heterologous transfer of tumours was a failure until the tumours became autonomous, *i.e.* until they became invasive or cancerous. With the attainment of autonomy, genetic and species barriers were eliminated and tumours transplanted to unrelated animals and foreign species would grow.

While the immunological nature of the reaction of a host to a homologous or heterologous tissue transplantation was widely recognised only comparatively recently, it has long been realised

that the basis of the differences between host and donor tissue was genetic. Loeb and Wright (1927) showed that inbred brother-sister lines of guinea-pigs could be rendered sufficiently homozygous to accept grafts from other individuals of the line, but not from animals of a second inbred line. F_1 hybrids would accept grafts from animals of either line, but grafts from the hybrids would not take in animals of either pure line. Studies with rats and mice (*e.g.* Little, 1941 ; Bittner, 1944) have given similar results.

The most effective attack upon the problem of tissue transplantation as a genetically controlled immunological process is that of Medawar (1945), who by making simultaneous " fitted " grafts from each of 25 heterozygous rabbits to the other 24, established that in these animals there were at least 127 " skin transplantation groups " governed by at least 7 antigens. In a subsequent paper Medawar (1946*b*) demonstrated that while rabbit's red blood cells cannot be used for immunisation against skin grafts, the leucocytes do share a number of antigens with skin tissue, and effective immunisation against homologous skin grafts can be obtained by the intradermal inoculation of suspensions of that rabbit's leucocytes. Medawar was unable to demonstrate antibodies by serological methods, but by making mitotic figure counts in transplanted autografts and first and second set homografts, he was able (Medawar, 1946*a*) to show that the antibody generated by skin homografts brought cell division in the grafts to a standstill.

Medawar and McDonald (1947) have recently investigated the genetic background of skin transplantation immunity in mice, using two strains, one of 30 and the other of 22 successive generations of brother-sister mating. Neither of these strains was genetically uniform so far as genes controlling skin antigens were concerned. As might be expected, skin transplanted between related, but not homozygous, mice can sometimes survive despite clear evidence of an immune response. Here presumably a single " foreign " antigen has failed to elicit a vigorous enough immunological response.

It is of interest to speculate on the basis of the immunological intolerance of foreign tissues. Professor Medawar tells us he was unable to demonstrate any damaging effect on tissue cultures of donor rabbit skin with sera cells or tissue extracts of rabbits heavily immunised by the repeated application of large grafts. *In vivo* his findings suggested that the effect of immunisation was to prevent the multiplication of the donor cells.

Harris (1943 *a* and *b*) carried out experiments with roller tube tissue cultures of various organs of rats and mice. He found that mixed cultures of rat and mouse cells survived with normal morphology in normal rat serum. If, however, serum from rats which had been previously immunised with mouse tissues was used in such mixed cultures specific destruction of the mouse cells resulted. Though these experiments differ in important respects from Medawar's they

may indicate when taken along with his that the immune reaction is not mediated by normal antibody, but an agent of the type that has been postulated for the production of tuberculin hypersensitivity. One might assume that the mononuclear inflammatory cells in the neighbourhood of the graft were specifically stimulated, by contact with the donor antigen against which they had been sensitised, to liberate the same pharmacologically active substance as is concerned in the tuberculin reaction, and that this substance is responsible for the inhibition of multiplication. The suggestion has at least the merit that it is amenable to an experimental test.

5. THE INFLUENCE OF GENETIC FACTORS ON THE IMMUNE RESPONSE

The only type of immunological reactivity in which the role of heredity has been extensively studied is *allergy* in man. Twin studies (Hanhart, 1940) revealed that of 71 pairs of identical twins there was an 80 per cent. concordance in respect of hay fever, 60 per cent. in respect of migraine and 28·6 per cent. in respect of asthma. Numerous investigations have been made of the role of heredity in human allergy, and a genetic analysis of the available data has been made by Wiener *et al.* (1937). When the age of onset of allergic disturbances (that is hay fever, asthma, urticaria or food allergy) is studied, two peaks are found, one in early childhood and one in the third decade of life. Wiener *et al.* suggested that the tendency to develop allergic disease is transmitted by a single pair of allelomorphic genes, H and h, where H denotes absence of allergy and h allergy. There are three genotypes—HH, who are quite normal, hh, who develop symptoms of allergy before puberty, and the heterozygotes Hh. The last are either normal transmitters (five-sixths) or develop allergic disease after puberty (one-sixth). One difficulty of Wiener's hypothesis, so far unexplained, is the observed excess of males over females in those developing allergic disease before puberty.

To us this interpretation seems unduly simplified. Many allergists consider that probably every individual has at some time shown allergic symptoms of one sort or another, and it is quite certain that the conditions of exposure to the sensitising substance play an important part in determining clinical sensitisation. There is no question that genetic factors are deeply concerned, but it seems highly improbable that the tendency to allergic disease depends on a variation in a single gene.

A few investigations have been made in animals. By selective breeding Chase (1941) was able to demonstrate that the susceptibility of guinea-pigs to skin sensitisation with simple chemical compounds was influenced by heredity. Using *d*-nitrochlorobenzene and poison ivy in succession, he found that there was a rough accordance between the sensitivity developed to the two compounds although there were

several instances of discrepancy. Similarly, Jacobs, Kelley, and Sommers (1941) were able to establish a strain of guinea-pigs, practically all members of which could be sensitised readily and strongly with allyl isothiocyanate, a compound which will sensitise only a small percentage of unselected animals.

It is common in experimental immunisation to find that one animal develops much higher antibody titres than another after similar courses of inoculations. There have been few investigations made to see whether the capacity to produce high titre or low titre antibodies to various types of antigens is controlled by genetic factors. One factor underlying this variation is the variable genetically controlled presence of the hapten concerned in the tissues of the laboratory animal used. For instance, Wheeler, Sawin, and Stuart (1939*a*) proved that the occurrence of A substance in the tissues of rabbits is inherited as a dominant Mendelian factor. Animals possessing A substance failed to produce α-agglutinins on the injection of human A-erythrocytes, but animals of an inbred strain lacking this character produced uniformly high titres. The same authors (1939*b*) found that the capacity of rabbits to produce antibodies for the M-antigen in human red cells appeared to be inherited; but here lack of ability to produce antibody could not be traced to the presence of M substance in the animals. Lewis and Loomis (1928) tested the members of four families of inbred guinea-pigs for their ability to form antibodies to cattle and sheep red blood cells, to *S. typhi*, and to *Br. abortus*. They found that when single small or moderate doses of antigen were used, so that the primary response only was observed, familial differences were clear-cut, two families being good, and two poor, antibody producers. With repeated doses or very large single doses, which involved the secondary response, the results were irregular.

The available evidence indicates that genetic constitution does play a part in deciding the efficiency of the immune response, but the position is complex and the reactions of the same animal to different antigens may differ greatly.

6. A THEORETICAL DISCUSSION OF ANTIBODY PRODUCTION IN THE LIGHT OF MODERN THEORIES OF GENE AND ENZYME ACTION

Any discussion of the application of immunological methods to genetic studies must be incomplete if it is limited, as previous reviews have been, merely to the provision by immunological methods of data about phenotypic structure not recognisable by other means. It is of greater interest, and of potentially greater practical importance, to attempt to elucidate the process by which foreign molecular patterns give rise to characteristic immunological responses, including sensitisation phenomena as well as production of classical serum antibody.

In most respects the discussion which follows is based on a monograph on " Production of Antibodies," published by one of us in 1941.

Fuller details of the experimental work on which it is based will be found in that monograph, a second edition of which is in preparation. The present approach is, however, along quite distinct lines, being largely influenced by the rapidly developing new conceptions of gene and enzyme action.

Our procedure will be to outline first what we regard as the relevant facts in regard to the physiology of antibody production, with special reference to the types of cells concerned in the process. This will be followed by an attempt to restate in somewhat modified form the concept of antibody production as an adaptive enzyme process. The argument will be almost wholly at the biological level and will centre in the conception that the cells concerned in antibody production have two important functions : (i) the removal of effete body cells and components and (ii) the removal of, and immunological response to, invading micro-organisms.

(a) The production of antibodies

There is more than one type of antibody. An anti-pneumococcal horse serum contains its specific activity almost wholly in the form of a globulin of high molecular weight, approximately 1,000,000, and of great asymmetry. Diphtheria antitoxin produced in the same species is predominantly a function of molecules similar to normal γ globulin and of molecular weight about 180,000. In man and rabbit nearly all antibodies are of this latter character, but even so there are considerable and perhaps fundamental differences from one type of antigenic response to another. Under the circumstances, it is extremely difficult to develop any general theory of antibody production. There is an almost infinite number of possible antigens and three different mammals—man, horse, and rabbit—whose immunological responses are of major practical or theoretical importance. Unfortunately, there is probably no single combination of antigen and antibody producer in which all the relevant points have been investigated. Our list of the aspects of the problem, which have to be covered by any adequate antibody theory, will necessarily be drawn from a rather wide range of antigenic responses in more than one host species. As far as possible they will be taken from examples in which " normal " antigenic stimuli have been used. There can be no reasonable doubt that the antibody mechanism is something which has been developed as an increasingly effective means of dealing with bacterial invasion. As such its efficiency has probably been maintained at a high level, since any breakdown will sharply diminish survival chances. It will presumably therefore function most normally when the antigen used as stimulus is a bacterial product such as toxin or toxoid, or bacterial bodies themselves.

Let us take the reactions observed in rabbits immunised with, say, staphylococcal toxoid on the one hand, and a bacterial vaccine on the

other, as standard, and where necessary use more convenient evidence from other systems. We may then state the essential characteristics of antibody production as follows :—

(1) Antibody is physically similar to the γ globulin of normal serum (or exceptionally to some other normal globulin), differing in that each molecule possesses one or more modified areas of surface which react specifically with certain molecular patterns of the corresponding antigen. The relation is a complementary one not yet expressible in physical terms, but legitimately pictured as a lock-key or die-coin relation.

Several types of evidence support the existence of antibody incapable of producing the usual aggregation reactions and detectable only by indirect means. It is usual to regard this antibody as univalent in the sense of having only one specifically reactive group per molecule.

(2) Antibody is produced only in response to the entry of antigens into the parenteral tissues of the body. Antigens are macromolecular or particulate in character ; most are protein or complexes of other types of substance with protein. It may be that all antigens are either protein or capable of combining with the protein of the antibody producer. It is still possible, however, that some complex biological molecules free from protein may function as antigens in their own right. In general, any protein (or other macromolecular substance) is antigenic if (*a*) it is not normally present in the animal immunised ; (*b*) it can be broken down into disposable units by the tissues of the animal ; and (*c*) it is not too rapidly eliminated from the body. There are wide variations in the ease with which antibodies are produced against different antigens.

(3) With " good " antigens the time aspect of antibody production varies characteristically according to the nature of the antigen. On the one hand, a *toxoid* given in a small to moderate dose provokes no antitoxic response, but a second similar inoculum given say 14 days later provokes a rapid sharp response beginning in 2-3 days and reaching a peak at 7 days. On the other hand, a *particulate antigen* gives rise to a response commencing 4-7 days after injection and reaching a peak at 10-14 days.

The reason for these differences is not fully determined. The most important difference is probably that the toxoid molecules are in a condition to be taken up immediately and almost simultaneously by the antibody-producing mechanism. Particulate antigens, *e.g.* bacteria, probably undergo a rather prolonged process of breaking down before the antigenically effective elements are available (*cf.* Burnet, 1941).

(4) With any type of antigen, an animal which has once been immunised responds more rapidly and effectively to a fresh stimulus with the same antigen than a normal animal.

(5) Antibody is produced by mesodermal cells including the interrelated types known as large and small lymphocytes, plasma cells, histiocytes and cells of the reticulo-endothelial system. Such

cells characteristically accumulate in the filtering mechanisms of the body, notably the lymph nodes and spleen, but also in the liver, bone marrow, and lungs. Lymphocytes appear to be a major source from which formed antibody (against bacteria) is liberated into the blood.

(6) Antibody in the circulation is being constantly removed at a rate proportional to its concentration with a "half-life" in some instances of about 2 weeks.

(7) Antibody production following an antigenic stimulus rises to a peak and then diminishes; but it continues at a diminishing rate for long periods. It seems to us that there is adequate evidence that antibody production continues after the antigen has been completely eliminated from the body. This, however, is by no means generally admitted. The theories of antibody production still current demand that antibody shall be produced only so long as the "antigenic determinants," at least of the antigen, remain in the body as a template for the production of the specific antibody pattern.

(8) Arising from (7) we must assume that antibody production is a function not only of the cell stimulated by contact with antigenic molecule or particle but also of its descendants. The average life of a lymphocyte is less than 24 hours.

(9) The antibody globulin molecules produced by a standard antigenic stimulus in a given species of animal are not uniform. They vary in two ways :—

(*a*) Absorption with related but not identical antigens will show that there are at least minor differences in specificity amongst the antibody molecules in the immune serum.

(*b*) The character of the antibody population differs according to the age of the animal and the intensity and time distribution of the antigenic stimulus.

(*b*) *The cells concerned in antibody production*

There is still controversy as to the actual cells involved in antibody production in mammals, but the competitors for this function are, so far as we are aware, limited to three types—macrophages (histiocytes, cells of reticulo-endothelial system), lymphocytes, and plasma cells. All these cells appear to be relatively undifferentiated descendants of the primitive mesenchymal cells (reticular cells) that are found in loose connective tissue, lymph nodes, spleen, etc.

The picture presented by histological textbooks (Maximow and Bloom) is of persisting relatively quiescent reticular cells which can, under appropriate conditions proliferate, producing either macrophages or medium or large lymphocytes. Both these types of cell are abundant; under ordinary circumstances they produce by mitosis cells like themselves, but the large lymphocytes can give rise to small lymphocytes or plasma cells; both of these appear to be end cells

not capable of further proliferation. There is some evidence that lymphocytes can, under certain circumstances, develop into macrophages. According to Maximow, the hæmocytoblast, from which all the red and white blood cells are derived, is indistinguishable from the medium lymphocyte, and the line of development which its progeny follow is determined essentially by internal environmental factors.

It is certainly within this closely interrelated group of cell types that antibody production occurs and, from the histological evidence of transitional forms, it would seem likely, first, that cells of slightly differing character within the group might all be responsible for antibody production under appropriate stimulus and, secondly, that the type of antibody produced might well differ according to the type or stage of specialisation of the cells responsible for its production.

It is necessary to recognise the existence of several different types of specific immunological reactivity each presumably mediated by what can legitimately be called antibody molecules. Those antibodies which provide, with the corresponding antigen, aggregation reactions of various sorts easily studied *in vitro* have been most intensively studied. For obvious reasons there is a tendency to centre all discussions of the nature and origin of antibodies in this particular type. This is legitimate enough provided the existence of other types of antibody, *e.g.* those concerned in the common allergic conditions of man, or responsible for tuberculin and similar types of sensitisation, are kept in mind. In fact it is only in regard to antibacterial circulating antibody, agglutinin or precipitin for specific polysaccharide, that we know enough to allow any useful discussion of origins.

Following any localised bacterial or virus infection, or any local injection of inactivated bacterial or virus cultures as a vaccine, antibody production is mainly a function of the lymph nodes draining the area. The evidence for this conclusion available in 1940 is fully reviewed by Burnet (1941). Since then two groups of American workers have made important contributions in support of the thesis. Ehrich and his colleagues at Philadelphia (Ehrich and Harris, 1942 ; Harris *et al.*, 1945 ; Ehrich *et al.*, 1946 ; Harris and Ehrich, 1946) have studied the response in rabbits to antigens injected into the hind foot, with particular reference to the part played by the popliteal lymph node. Their experiments justify the conclusion that a large proportion of the antibody that eventually appears in the blood is produced in the lymph node which it leaves in the substance of lymphocytes, such as can be collected in the efferent lymph channel. When the vaccine was emulsified in paraffin oil the antibody response was enhanced, and concomitantly there was a marked accumulation of monocytic macrophage cells around the site of injection. These showed no excess of antibody, and again the evidence pointed to antibody production in the lymph node as being of major importance.

The second group of workers, Dougherty, White and colleagues at New Haven (Dougherty and White, 1945; Chase, White, and Dougherty, 1946), have approached the problem as physiologists interested in endocrine reactions. Their thesis is that lymphocytes are essentially storehouses of certain substances which are discharged into lymph and blood streams when needed. The discharge is accomplished by budding or dissolution of the cytoplasm with associated nuclear degeneration. The stimulus to discharge is the existence in the circulation of an effective concentration of adrenal cortical hormone. Experimentally the stimulus can be provoked by subcutaneous injection of either adrenal cortical extracts or the adrenotrophic hormone of the pituitary. Dougherty and White have shown that a protein of γ globulin type can be obtained from lymphocytes, that there is marked disappearance of lymphocytes an hour or two after injection of appropriate hormones, and that this is associated with an increase in β and γ globulin content of the serum and, in appropriately immunised animals, with an increase in circulating antibody titre.

The two sets of findings are mutually consistent and in agreement with the findings of earlier workers. Any theory of antibody production must therefore be able to account for the special characteristics of this process taking place in the lymph nodes draining the area in which antigen was deposited.

There are three major histological characteristics of a lymph node which are relevant to the present discussion.

(1) The multiple afferent lymph vessels discharge into the cortical sinuses which are lined by numerous macrophages appropriately situated to take up micro-organisms or other foreign material reaching the node. As shown by Drinker, Field, and Ward (1934) the lymph node is a very effective bacterial filter.

(2) Dense nodules with (usually) germinal centres are distributed through the cortex of the node. These appear to represent the chief sites of lymphocyte production. The centres are filled with paler staining medium and large lymphocytes, reticular cells and macrophages. At the periphery there are densely packed small lymphocytes. Other accumulations of lymphocytes form the medullary cords, and from both sources small lymphocytes pass to the sinuses and leave the node by the efferent vessels emerging at the hilum.

(3) Phases of development and depletion of nodules occur and are presumably associated with the pituitary-adrenal endocrine mechanism described by Dougherty and White. In the phase of depletion there is evidence of marked destruction of lymphocytes.

The basic facts on which the interpretation of antibody production in lymph nodes must be based are (i) the phagocytosis of particulate antigenic material by macrophages which line the sinus spaces, (ii) the presence of antibody in (small) lymphocytes emerging from the node, and (iii) the fact that small lymphocytes are being constantly produced

and destroyed. Any theory of antibody production which postulates the continuing presence in the antibody-producing cells of a template of antigen (or antigenic determinant) against which the antibody globulin is synthesised, must find an insuperable difficulty in the characteristically short life of the lymphocyte and the macrophage. The intense reactivity of a lymph node to toxic and hormonal influence with rapid multiplication and apparently almost catastrophic destruction of cells, provides no reasonable place for cells which can hold antigen and for weeks or months go on producing globulin molecules moulded on its specific pattern.

We would stress again that this discussion of the part played by lymphocytes in antibody production is based only on the response to parenteral injection or invasion of particulate antigens like foreign red cells and bacteria. It may be and probably is quite inapplicable to the production of antitoxins or to the development of various types of sensitisation.

(c) *The biological basis of immunological response*

The system of mesenchymal cells concerned with the production of antibody has many functions in the body, but in broad terms these functions can be reduced to three: (1) the disposal of effete body cells and constituents, (2) phagocytosis and destruction of invading micro-organisms, and (3) the production and perhaps transportation of antibody. These functions we may now consider.

(1) The best known type of expendable cell in the body is the red blood cell. It is usually estimated that the maximum life of a red cell in the human circulation is about 120 days, 50 ml. of cells being replaced daily. Effete cells are removed predominantly by macrophage cells in the splenic pulp. Lymphocytes as has been mentioned have much shorter average life, but their fate is more obscure. According to the theory of Dougherty and White (1945), under the stimulus of an increased circulating concentration of adrenal cortical hormones, lymphocytes liberate γ-globulin by shedding cytoplasm. The degenerating nuclei in lymph nodes are largely phagocytosed by macrophages, and it is reasonable to hold that this is also the fate of lymphocytes which have outlived their function in other parts of the body. In the repair of traumatised tissues damaged material of all types is normally dealt with by histiocytic cells of the macrophage type.

(2) The primary response to acute invasion by pathogenic micro-organisms is predominantly by polymorphonuclear leucocytes. Macrophage activity is more obvious with subacute or chronic infections, but even with acute infections the macrophage is concerned both with direct phagocytosis of micro-organisms and with the final disposal of leucocytes which have been damaged by taking up particulate or soluble bacterial material. The nature of the process of inflammation, destruction of pathogenic micro-organisms and

repair is highly complex and varies considerably from one type of infection to another. Irrespective of complications, however, it appears to be generally accepted that the final fate of the substance of micro-organisms successfully resisted, as of the substance of damaged body cells, is reduction to its proximate constituents within macrophage cells and subsequent passage of these via the blood plasma to the general metabolic activities of the body.

(3) Looked at from the point of view of an adaptive mechanism, antibody production is a means to favour survival (*a*) by providing a more efficient means of countering the later stages of the micro-organismal infection that invoked the response, and (*b*) to ensure that a future attack by the same micro-organism can be resisted effectively from the moment that infection is initiated. Again, the process is complicated and variable, but there can be no doubt about its importance as a factor of survival in an environment replete with potential pathogens. Nor is there any evidence that immunological reactivity has been evolved to favour survival by any other function than this provision of more effective means of dealing with microbic invasion. Most of the other circumstances by which immunological phenomena are invoked are grossly artificial, blood transfusions, intravenous or subcutaneous injections, skin grafting and so on—and the resulting phenomena must be interpreted as the automatic responses of a mechanism "designed" for one purpose to an effective but "unreasonable" stimulus to which no biologically appropriate reaction is possible.

There are two current hypotheses as to how typical antibody is produced in mammals. The first, usually known as the Haurowitz-Mudd hypothesis, has been elaborated in detail by Pauling (1940) and is accepted by the majority of authors as essentially correct. It assumes that antibody is γ-globulin that has been spatially patterned at certain points—at the two ends of a bipolar molecule according to Pauling—by its synthesis in contact with the antigen molecule which acts as a template. The theory therefore demands that antibody is produced only so long as the essential antigenic determinants remain present in the antibody-producing cells. This has never been directly demonstrated, and for reasons given in detail in a previous discussion (Burnet, 1941) and summarised in previous sections of this paper, we consider that the weight of evidence is strongly against the persistence of antigen for the very long periods over which circulating antibody may be present.

The second hypothesis due to Burnet (1941) is that antibody production must be regarded as in many ways equivalent to the development of an adaptive enzyme process. On this view immunological phenomena derive essentially from the intrusion of a foreign pattern into that nest of uniquely patterned and mutually tolerant enzymes, the living substance of the cell. From practically every type of actively metabolising cell there can be extracted enzymes

capable of breaking down each of the major types of substance, protein phospholipid and polysaccharide, characteristic of living matter. Within the living functioning cell their mutual destructiveness is curbed by some means which we cannot particularise, but which must be related in some way to their respective molecular patterns and to their spatial relationship to one another. Omitting for the time being the problem of the disposal of damaged cells or cell fragments of the body's own pattern, we assume that when an alien molecule or particle finds itself in such an environment it is somehow recognised as "not-self" and is subject to destruction by appropriate enzyme systems.

Adaptive enzyme systems are well known in bacteria and yeasts, and in one instance direct evidence has been obtained that an adaptive enzyme system once established can replicate itself indefinitely (provided the substrate is continuously present) independently of any nuclear genetic control (Spiegelman, Lindegren, and Lindegren, 1945).* Such an ability of cytoplasmic entities to replicate themselves in accordance with appropriate internal environmental stimuli is now being explicitly or tacitly accepted in a considerable range of biological phenomena, *e.g.* Billingham and Medawar's (1947 *a* and *b*) work on the transfer of pigmentation in guinea-pig skin and that of Postgate and Hinshelwood (1946) on adaptive enzyme processes in *Esch. coli mutabile*. It is in line with the view developed particularly by Beadle (1945) but foreshadowed by Haldane (1942) and Wright (1941) that each gene controls a particular enzymic reaction and that in the last analysis the population of enzyme molecules concerned may represent the "descendants," by a process of replication or partial replication, of the gene itself. This view may be an unjustifiably wide extension of the conditions observed in regard to biochemical mutations in *Neurospora*, but it seems highly likely to be applicable to such situations as those concerned with antibody production.

The essentials of our hypothesis can be stated as follows. For reasons to be discussed in the next section, the enzyme systems concerned in the breakdown of antigenic particles or molecules require a certain degree of adaptive modification to function effectively against the foreign molecular pattern. Once this adaptation has occurred it is retained, persisting with replication of the enzyme system concerned. Possibly, as an immediate sequel to adaption and certainly after a recurrence of the same antigenic stimulus, replication of the system is greatly increased. If the cells concerned multiply or are converted into other types the modified enzyme systems are transmitted to their descendants. The appearance of antibody in the body fluids is to be regarded as a liberation, whether

* More recently Lindegren and Lindegren (1946) reported that they had been unable to repeat the experiments upon which the self-duplicating enzyme hypothesis was based. Repetition of experiments involving rare mutations is often very difficult, and final appraisal of the situation in yeasts must await further investigations.

by cytoplasmic dissolution or otherwise, of partial replicas retaining the adsorptive pattern, but not necessarily the disintegrating capacity of the modified enzyme systems.

The behaviour of one specialised type of immunological response, the development of tuberculin hypersensitiveness in infected animals strongly suggests that a type of antibody exists which can be transferred to other cells and confer on them the capacity of producing more antibody. If this is fully substantiated the possibility must be retained that complete replicas of the postulated enzyme systems can be transferred to other cells than those in which they were developed. We would suggest as one interpretation of the part played by the lymphocyte that there may be a transference of antibody-producing capacity from macrophages to the lymphocytes, which are always adjacent in the major sites of antibody production.

This general picture of antibody production is admittedly speculative, but we claim that it provides a reasonable interpretation of all the known characteristics of antibody production, including those which, in our view, render the Haurowitz-Mudd-Pauling theory untenable. Further, it is a view which seems to "fit" far better into the modern picture of intracellular processes that is being developed by biochemists and geneticists than the older theory.

The implications of the hypothesis for the genetic aspects of immunology are considered in the next section.

7. GENE-ANTIGEN RELATIONSHIPS

In practically every example that has been adequately studied, each antigenic pattern that is characteristic of the cells of some individuals and not others within a species, is inherited as if its character were controlled by a single gene. This has led to the suggestion, supported by Haldane (1942), Sturtevant (1944) and others, that the molecular pattern responsible for antigenic specificity might be traced back to the gene itself, and that in the last analysis the "code" by which phenotypic characters are represented in the chromosomal mechanism is equivalent in some way to the antigenic determinants which confer specific serological differences, *e.g.* between two of the artificial azoproteins of Landsteiner.

We feel that this may be an unduly sweeping generalisation. So far as the characters used in such studies of genetic material as we have described in earlier sections are concerned, a considerably simpler conception seems adequate.

The problem of antigenic individuality is most pressing in the field of reactions within the same species as exemplified by the production of red cell agglutinins and "blocking antibodies," or the intolerance of skin grafts from donors not isogenic with the recipient. At first glance there is a suggestion that almost every significant macro-molecule in the body may have an individuality that marks

it off from similar macro-molecules in other individuals. But in fact there is good evidence that between any two individuals of a species common molecular patterns are much more frequent than antigenetically dissimilar ones. There are strains of mouse carcinoma for instance which can be readily transferred from one mouse to another in heterogeneous stocks without provoking regression, and the practicability of corneal transplantation in man would indicate a low grade only of specificity in the tissues concerned.

All that the available data make it necessary to postulate is that a well-defined mechanism has been evolved to allow a differentiation of the response of macrophage and similar cells to damaged or effete body constituents on the one hand, and to foreign organic matter (always micro-organismal under natural conditions) on the other. It is essential for obvious reasons that the first should not provoke the antibody response appropriate to the second.

It is therefore necessary to endow each scavenging or antibody-producing cell with a capacity to " recognise " as foreign any potential antigenic determinants which are not represented in the body cells that it is liable to have to deal with under physiological or emergency conditions. Some qualification to this statement will probably be necessary to account for the special characteristics of the central nervous system and the chambers of the eye. Both situations are morphologically and physiologically shielded from concern with significant, *i.e.* non-fatal, emergencies, and their tolerance of implanted foreign tissues is probably in some way related to this characteristic.

Confining ourselves to the scavenging cells of the non-nervous tissues, unless we are prepared to admit an extraordinarily complex " code " by which such recognition of foreign structure is made, we are driven to the conclusion that all body cells carry some or all of a relatively small number of *marker* components whose specific character is determined by a correspondingly small number of genes. To account for the existence of individual differences, we must probably postulate a rather high mutation rate in these genes. The markers in question are not uniformly distributed to each type of cell. Medawar (1946*b*) has shown for instance that red cells from a donor animal will not " immunise " the recipient, so as to produce an accelerated response against a skin graft from the same donor, but that a leucocyte suspension will do so. This would indicate that there are different " markers " in red cells from those in leucocytes.

On this view there is within each phagocytic cell of the reticulo-endothelial system a mechanism tuned to respond to any " self-marker " in material which it takes into its cytoplasm, by a non-immunological destructive process.

If organic material which contains none of the molecular patterns characteristic of the self-markers enters the cell, the latter responds by the development of the adaptive mechanism we have described, so initiating antibody production. In the embryonic animal, and

probably in the cells associated with the central nervous system, this mechanism appears to be absent or modified.

It seems likely that antibody production is most effective when substances are present in the ingested foreign material, which are of the same general type as one of the self-markers but have a sharply distinct pattern. The mucopolysaccharide-protein complex carrying the ABO blood group, and also present in a wide variety of mucus-secreting cells, is almost certainly one such marker. It is well known that the somewhat similar polysaccharide complexes present on the surface of most bacteria are particularly potent antigens.

This hypothesis may be put somewhat differently. Within the potential antibody-producing cells there are enzymic groups adapted genetically to "fit" a sufficient number of marker constituents to allow differentiation of "self" from "foreign" (*i.e.* normally invasive micro-organismal) organic material. When such a "fit" occurs, normal disintegration processes go on without stimulation to immunological activity. An antigen is such in virtue of the fact that it corresponds in its general chemical character to one or other of the self-markers, but will only fit the corresponding enzyme when this is deformed to an appropriate adaptive configuration. It is this *deformation* which provides the stimulus for replication and antibody liberation.

On this view the most effective antigenic stimulus will be from an antigen of general type closely corresponding to a marker but sufficiently distinct to demand a considerable adaptive deformation of the enzyme. Poor antigens may be either too close in character to the marker or so distant that only under unusual conditions can any type of contact with one of the enzymes be made. Where the material presented is of wholly distinct chemical type, *e.g.* racemised protein, no antibody is produced.

The difference between the effectiveness of primary and secondary antigenic stimuli, for which no explanation seems to be available on the Haurowitz-Mudd theory, requires some discussion. The present theory would assume on general biological analogies that a gradual reversion of the modified enzyme systems toward the original marker configuration would occur. The modified system would, however, for a long period remain particularly suited to react with represented specific antigen and would, moreover, be present in greater numbers as a result of the primary antigenic stimulus. There is no evidence to decide whether a new deformation is necessary to stimulate replication and antibody liberation, or whether the adapted mechanism responds by such replication to fresh contact with antigen irrespective of whether deformation is or is not necessary. What appears to be certain is that prolonged re-stimulation does result in modification of the antibody produced, and by hypothesis, therefore, deformation of the originally modified enzyme.

The use of adjuvants such as paraffin oil emulsion to intensify

the antigenic response to a poor antigen would on our present hypothesis depend on the existence of a prolonged uniform flow of antigen to the cells. A poor antigen will only rarely find an opportunity to make effective contact with the marker enzyme most nearly appropriate. But if as a result of a few effective contacts replication of such modified, or partially modified, enzymes occurs, antigen arriving later will find more and more opportunity of making effective contact leading eventually to active antibody production.

Reverting now to the significance of genetically determined antigenic differences between related vertebrates, our conclusion would be that most of the differences that it is practical to study concern marker substances. Since of all cells the red blood corpuscles are those most extensively destroyed by cells of potential antibody-producing capacity, markers for the components of the red cells are specially abundant. This, plus the convenience of experimental studies on the cells, are probably the reasons for the effective attack on genetic problems that has been possible in this field.

CONCLUSION

It must have been evident from this review that despite a promising beginning it will be long before a balanced integration of the mutually relevant aspects of immunology and genetics will be possible. At the present time, for reasons of practical importance and technical convenience, comprehensive data are practically confined to red cell antigens of vertebrates with minor contributions from work on the transplantation of normal and malignant tissues.

As far as red cell antigens are concerned it appears to be a legitimate generalisation that immunological differences of a single group of antigens within a species correspond to the different allelomorphs of a single gene. Further it seems that, in some way analogous to the existence of the appropriate α or β agglutinins in human beings of different blood groups, the reticulo-endothelial cells of the vertebrates contain enzyme systems genetically " tuned " to the antigens of the body cells which it is their function eventually to destroy. This concept of " self-marker " groupings of expendable body cells as a necessity to allow immunological or non-immunological response as required, is the main novelty in the present review.

The general theory of antibody formation that we have adopted is not accepted by immunologists generally. This may be due either to the inadequacy of evidence for the theory or because active work in immunology during recent years has been predominantly from the biochemical or physical-chemical point of view. We hope, however, to have shown that at least some such approach is necessary if immunological phenomena are to be integrated into the developing pattern of general biology.

Finally, we would attempt a few suggestions as to fields from which

valuable help toward the construction of a real science of immuno-genetics might come :—

(i) The first is from a combined biochemical, genetic, and immunological study of some convenient bisexual micro-organism such as *Neurospora*. This would eliminate the complications inherent in the use of vertebrate cellular antigens.

(ii) Either in connection with such a study or with other material the possibility of recognising specific enzyme molecules by immunological means should be explored. An antiserum specific for an enzyme present in the parent stock but not in a genetically defined mutant might become a most potent weapon for the development of genetic theory.

(iii) A close study of the limitations of immunological response in the sheltered tissues of the mammal, especially to the central nervous system and the eye, would probably throw much light not only on the basis of immunity but on some important medical problems, *e.g.* the etiology of multiple sclerosis.

(iv) There are interesting possibilities from experiments now technically possible, in which tissues which have been removed and stored in the cold are returned to the same donor animal after it has been subjected to some type of immunising or sensitising manipulation.

(v) Lastly there are still opportunities to be exploited in the immunological study of human identical twins particularly in regard to the details of allergic sensitivity.

SUMMARY

1. An outline is given of the general techniques and concepts of immunology as applied to genetic problems.

2. Research in immuno-genetics has been dominated by the necessity for dealing with practical problems arising out of the clinical use of blood transfusion and for this and other reasons most work has been concerned with the antigens of red blood cells. A summarised account of the conclusions in regard to the red cell antigens of mammalian and avian species is given.

3. Another important field also with immediate surgical implications is concerned with the reaction to tissue transplants from other individuals of the same species. The reaction against such transplants is of immunological character.

4. Without established exception there appears to be a one gene-one antigen correspondence.

5. The use of immunological techniques in the study of genetic problems has up to the present merely provided another means of recognising differences between two individuals and of elucidating the mechanism of inheritance of such differences. It is the main object of this review to stress that immunological phenomena have an interest for the geneticist at a much deeper level. Antibody

production and the other manifestations of acquired immunity and specific sensitisation represent the most clear-cut examples of induced alteration in the chemical mechanisms of the organism. An attempt is made to present a biological interpretation of these phenomena in the form of a restatement of an earlier discussion by Burnet (1941) on the production of antibodies.

The main features of this interpretation are: (1) Antibody production is dependent on processes analogous to those concerned in the development of adaptive enzymes in bacteria; (2) the intracellular unit (enzyme?) modified by effective contact with the antigen is capable of replication and probably of transfer to other cells; (3) circulating antibody molecules are regarded as partial replicas of the intracellular units; (4) the production of antibody is not dependent on the continuing presence of antigen in the body.

6. A consideration of the cells involved in dealing with foreign organic material and in the production of antibody shows that these are the same cells as are concerned with removing and destroying effete or damaged body cells. No antibody is produced against components of such cells except perhaps under rare pathological conditions. The hypothesis is presented that differentiation between foreign and "self" components by cells of the reticulo-endothelial system depends on the existence of certain "marker" components on all expendable body cells. The number of markers is probably small and their character determined by a correspondingly small number of genes. Only a limited range of biologically significant molecules are "good" antigens, and there is evidence which suggests that only substances whose general chemical structure resembles that of one of the "markers" readily produce antibody. The differences observed by immunological methods amongst red cells may all be due to marker components.

7. In the concluding section a number of suggestions are made as to directions in which experimental studies might be developed if the outlines of the theoretical picture of immunogenetics which we have presented are to be filled in.

REFERENCES

AMINOFF, D., MORGAN, W. T. J., AND WATKINS, W. M. 1946. Specific serological characters of the mucoids of hog gastric mucin. *Nature 158*, 879.

BEADLE, G. W. 1945. Biochemical genetics. *Chem. Rev. 37*, 15.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1947*a*. The "cytogenetics" of black and white guinea-pig skin. *Nature 159*, 115.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1947*b*. The role of dendritic cells in the infective colour transformation of guinea-pig skin. *Nature 160*, 62.

BITTNER, J. J. 1944. The transplantation of splenic tissue in mice. *Pub. Health Rep. 51*, 244.

BURNET, F. M. 1934. Antigenic differences between related bacterial strains: a criticism of the mosaic hypothesis. *Brit. J. exp. Path. 15*, 354.

BURNET, F. M. 1941. *The production of antibodies*. Walter and Eliza Hall Inst. Monograph 1. Melbourne: Macmillan.

CHASE, M. W. 1941. Inheritance in guinea-pigs of the susceptibility to skin sensitisation with simple chemical compounds. *J. exp. Med. 73*, 711.

CHASE, J. H., WHITE, A., AND DOUGHERTY, T. F. 1946. The enhancement of circulating antibody concentration by adrenal cortical hormones. *J. Immunol. 52*, 101.

COOMBS, R. A., MOURANT, A. E., AND RACE, R. R. 1945. A new test for the detection of weak and " incomplete " Rh agglutinins. *Brit. J. exp. Path. 26*, 255.

CUMLEY, R. W., AND IRWIN, M. R. 1942. Individual specificity of human serum. *J. Immunol. 46*, 63.

CUMLEY, R. W., IRWIN, M. R., AND COLE, L. J. 1943. Genetic control of species specific antigens of serum. *J. Immunol. 47*, 35.

DOUGHERTY, T. F., AND WHITE, A. 1945. Functional alterations in lymphoid tissue induced by adrenal cortical secretion. *Amer. J. Anat. 77*, 81.

DRINKER, C. K., FIELD, M. E., AND WARD, H. K. 1934. The filtering capacity of lymph nodes. *J. exp. Med. 59*, 393.

EHRICH, W. E., AND HARRIS, T. N. 1942. The formation of antibodies in the popliteal lymph node of rabbits. *J. exp. Med. 76*, 335.

EHRICH, W. E., HARRIS, T. N., AND MERTENS, E. 1946. The absence of antibody in the macrophages during maximum antibody formation. *J. exp. Med. 83*, 373.

FERGUSON, L. C. 1941. Heritable antigens in the erythrocytes of cattle. *J. Immunol. 40*, 213.

FERGUSON, L. C., STORMONT, C., AND IRWIN, M. R. 1942. On additional antigens in the erythrocytes of cattle. *J. Immunol. 44*, 147.

GIBSON, T., AND MEDAWAR, P. B. 1943. The fate of skin homografts in man. *J. Anat.*, London, *77*, 299.

GREENE, H. S. N. 1941*a*. Heterologous transplantation of mammalian tumors. I. The transfer of rabbit tumors to alien species. *J. exp. Med. 73*, 461.

GREENE, H. S. N. 1941*b*. Heterologous transplantation of mammalian tumors. II. The transfer of human tumors to alien species. *J. exp. Med. 73*, 474.

GREENE, H. S. N., AND MURPHY, F. D. 1945. The heterologous transplantation of mouse and rat tumors. *Cancer Res. 5*, 269.

GORER, P. A. 1938. The antigenic basis of tumor transplantation. *J. Path. and Bact. 47*, 231.

GORER, P. A. 1942. The role of antibodies in immunity to transplanted leukemia in mice. *J. Path. Bact. 54*, 51.

HALDANE, J. B. S. 1942. *New paths in genetics*. London : Allen and Unwin.

HANHART, E., IN BERGER, W., AND HANSEN, K. " *Allergie*." Leipzig : Thieme. 1940.

HARRIS, M. 1943*a*. The compatibility of rat and mouse cells in mixed tissue cultures. *Anat. Rec. 87*, 107.

HARRIS, M. 1943*b*. The role of humoral antagonism in heteroplastic transplantation in mammals. *J. exp. Med. 93*, 131.

HARRIS, T. N., AND EHRICH, W. E. 1946. The fate of injected particulate antigens in relation to the formation of antibodies. *J. exp. Med. 84*, 156.

HARRIS, T. N., GRIMM, E., MERTENS, E., AND EHRICH, W. E. 1945. The role of the lymphocyte in antibody formation. *J. exp. Med. 81*, 73.

HIRSZFELD, L. 1947. The transition forms of blood groups. *J. Immunol. 55*, 141.

HIRSZFELD, L., AND AMZEL, R. 1940. Etude sur les pléiades' isozériques' du sang. *Ann. Inst. Pasteur 65*, 251, 386.

IRWIN, M. R. 1946. Antigens, antibodies and genes. *Biol. Rev., 21*, 93.

IRWIN, M. R., AND CUMLEY, R. W. 1943. Immunogenetic studies of species relationships. *The American Naturalist 77*, 211.

IRWIN, M. R., AND CUMLEY, R. W. 1945. Suggestive evidence for duplicate genes in a species hybrid in doves. *Genetics 30*, 363.

JACOBS, J. L., KELLEY, J. J., AND SOMMERS, S. C. 1941. Hereditary predisposition to sensitization in guinea pigs. *Proc. Soc. exp. Biol. N.Y. 48*, 639.

LANDSTEINER, K. 1921. Ueber heterogenetisches antigen und hapten. XV. Mitteilung uber antigene. *Biochem. Ztschr. 119*, 294.

LANDSTEINER, K. 1928. Cell antigens and individual specificity. *J. Immunol.* *15*, 589.

LANDSTEINER, K. 1946. *The specificity of serological reactions.* Cambridge, Mass. : Harvard University Press.

LANDSTEINER, K., AND WITT, D. H. 1926. Observations on the human blood groups. Irregular reactions. Isoagglutinins in sera of group IV. The factor A^1. *J. Immunol. 11*, 221.

LANDSTEINER, K., AND VAN DER SCHEER, J. 1936. On cross reactions of immune sera to azoproteins. *J. exp. Med. 63*, 325.

LEVINE, P., AND STETSON, R. E. 1939. An unusual case of intra-group agglutination. *J. Amer. med. Assoc. 113*, 126.

LEWIS, P. A., AND LOOMIS, D. 1928. Allergic irritability. IV. The capacity of guinea pigs to produce antibodies as affected by inheritance and as related to familial resistance to tuberculosis. *J. exp. Med. 47*, 437.

LINDEGREN, C. C., AND LINDEGREN, G. 1946. The cytogene theory. *Cold Spring Harbor Symposia. Quant. Biol. XI*, 115.

LITTLE, C. C., IN SNELL, G. D. *The biology of the laboratory mouse.* Philadelphia. 1941.

LOEB, L. 1945. *The biological basis of individuality.* Springfield : Charles C. Thomas.

LOEB, L., AND WRIGHT, S. 1927. Transplantation and individuality differentials in inbred families of guineapigs. *Amer. J. Path. 3*, 251.

MAXIMOW, A. A., AND BLOOM, W. 1942. *Textbook of histology.* 4th ed. Philadelphia and London.

McGIBBON, W. H. 1944. Cellular antigens in species hybrids in ducks. *Genetics 29*, 407.

MEDAWAR, P. B. 1944. The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat.*, London, *78*, 176.

MEDAWAR, P. B. 1945. A second study of the behaviour and fate of skin homografts in rabbits. *J. Anat.*, London, *79*, 157.

MEDAWAR, P. B. 1946*a*. Immunity to homologous grafted skin. I. The suppression of cell division in grafts transplanted to immunized animals. *Brit. J. exp. Path. 27*, 9.

MEDAWAR, P. B. 1946*b*. Immunity to homologous grafted skin. II. The relation between antigens of blood and skin. *Brit. J. exp. Path. 27*, 15.

MEDAWAR, P. B., AND McDONALD, D. J. 1947. (Personal communication from P. B. Medawar.)

MORGAN, W. T. J. 1947. The human ABO blood group substances. *Experientia* III, 7.

MORGAN, W. T. J., AND WADDELL, M. B. R. 1945. A specific blood group O substance. *Brit. J. exp. Path. 26*, 387.

MORGAN, W. T. J., AND WATKINS, W. M. 1945. The examination of rabbit anti-A immune serum produced by means of an artificial A-antigen. *Brit. J. exp. Path. 26*, 247.

OWEN, R. D. 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science 102*, 400.

OWEN, R. D., STORMONT, C., AND IRWIN, M. R. 1947. An immunogenetic analysis of racial differences in dairy cattle. *Genetics 32*, 64.

PAULING, L. 1940. A theory of the structure and process of formation of antibodies. *J. Amer. chem. Soc. 62*, 2643.

POSTGATE, J. R., AND HINSHELWOOD, C. N. 1946. The adaptation of *B. lactis aerogenes* and *B. coli mutabile* and various carbohydrates. *Trans. Farad. Soc. 42*, 45.

RACE, R. R. 1944. An " incomplete " antibody in human serum. *Nature 153*, 771.

RACE, R. R. 1946. The Rh blood groups. *Schweiz. Med. Wchnschr. 37-38*, 921.

SNYDER, L. H. 1946. Medical genetics and public health. *Bull. New York Acad. Med. 22*, 566.

SPIEGELMAN, S., LINDEGREN, C. C., AND LINDEGREN, G. 1945. Maintenance and increase of a genetic character by a substrate cytoplasmic interaction in the absence of the specific gene. *Proc. Nat. Acad. Sci. 31*, 95.

STURTEVANT, A. H. 1944. Can specific mutations be induced by serological methods? *Proc. Nat. Acad. Sci. W.S. 30*, 176.

TATUM, E. L., AND LEDERBERG, J. 1947. Gene recombination in the bacterium *Escherichia coli*. *J. Bact. 53*, 673.

TODD, C. 1930. Cellular individuality in the higher animals, with special reference to the individuality of the red blood corpuscle. *Proc. Roy. Soc.* " *B* " *106*, 20.

TODD, C., AND WHITE, R. G. 1910. On the hæmolytic immune isolysins of the ox and their relation to the question of individuality and blood relationship. *J., Hyg.*, Camb., *10*, 185.

WHEELER, K. M., SAWIN, P. B., AND STUART, C. A. 1939*a*. Group specific agglutinins in rabbit serum for human cells. V. Inheritance of the A character. *J. Immunol. 36*, 349.

WHEELER, K. M., SAWIN, P. B., AND STUART, C. A. 1939*b*. Group-specific agglutinins in rabbit serums for human cells. VI. Immune specific M-agglutinins. *J. Immunol. 37*, 159.

WIENER, A. S. 1943. *Blood groups and blood transfusion*. Springfield : Charles C. Thomas.

WIENER, A. S., AND SONN-GORDON, E. B. 1947. Preparation of anti-Rh serum. *Amer. J. clin. Path. 17*, 67.

WIENER, A. S., SONN, E. B., AND POLIVKA, H. R. 1946. Heredity of Rh blood types. V. Improved nomenclature ; additional family studies with special reference to Hr. *Proc. Soc. exp. Biol. N.Y. 61*, 382.

WIENER, A. S., ZIEVE, I., AND FRIES, J. H. 1937. The inheritance of allergic disease. *Ann. Eugenics 7*, 141.

WRIGHT, S. 1941. The physiology of the gene. *Physiol. Rev. 21*, 487.

SENSITIVITY TO CO_2 IN *DROSOPHILA*— A REVIEW

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1. FOREWORD

THE inherited CO_2 sensitivity in *Drosophila* was discovered in 1938 in an ebony stock of *D. melanogaster* (Ph. L'Héritier and G. Teissier, 1938 and 1939). Normal so-called resistant flies can be kept for hours in an atmosphere of pure CO_2 without permanent injury. When returned to air they quickly recover from the narcosis and return to completely normal behaviour. With flies from the so-called sensitive stock quite different phenomena are observed. CO_2 is very dangerous for them and an exposure of a few seconds to this gas can poison them irreversibly. As with resistant flies, a sort of awakening follows the narcosis, but the sensitive flies remain injured permanently and display a more or less complete paralysis. If they are able to move their legs or wings, they do it in an unco-ordinated convulsive way which is very characteristic of the intoxication. They may succeed in recovering slowly, chiefly when kept in a moist and warm atmosphere ; but most of them die quickly. Except in very rare cases it is easy to distinguish a sensitive from a resistant fly.

Soon after the discovery of the phenomenon, the following facts were established. In crosses the sensitivity behaves as a hereditary unit, but, surprisingly enough, is not transmitted in a Mendelian fashion and exhibits no linkage with any chromosome. It seems to be bound to some discrete cytoplasmic agent which, because of its similarity with a chromosomal gene, was called a " genoid." An outstanding feature of this cytoplasmic inheritance is the fact that it is not purely maternal, for the genoid can be transmitted by the spermatozoon. Even supernumerary spermatozoa can sometimes introduce the sensitivity into the eggs of a resistant female (Ph. L'Héritier and G. Teissier, 1945).

This work was interrupted by the war and the sensitive stock was lost at the time of the German invasion of France in 1940. This would have been, the writer feels, a priceless loss for himself and perhaps for genetics in general. He is therefore very much indebted to Dr H. J. Muller, who carried the stock to America at the beginning of the war and had it preserved at Cold Spring Harbor. This allowed the work to start again in France in 1942 under poor war conditions. It was then found that the genoids, both in the somatic and the germ cells, are greatly affected by the temperature. Raising the temperature during different stages of development leads to permanent or temporary cures of sensitivity and also to the cure of the germ cells. The details of these phenomena are rather intricate and not yet entirely understood (Ph. L'Héritier and A. Sigot, 1946).

In 1946, the use of the techniques of organ transplantation of Ephrussi and Beadle (1936) led to the important discovery that the genoid is contagious and can invade the host and its germ cells from a transplanted organ. Soon afterwards, evidence was brought forward for the presence of the infectious agent in the hæmolymph and in the supernatant of ground and centrifuged sensitive individuals. These facts raised the question of the similarity between the genoid and a virus and allowed its study by techniques appropriate to virus research, such as ultrafiltration (Ph. L'Héritier and F. Hugon de Scoeux, 1947).

It thus became a simple matter, by injection into the abdomen, to turn resistant flies into sensitive and, since the genoids subsequently get into the germ cells, any stock of *D. melanogaster* and even of other species can be made sensitive. A new field of investigation was then opened, bearing on the possible relation between the chromosomic genotype and the manifestation as well as the inheritance of sensitivity. It remains still scarcely explored.

The singular importance of the inherited CO_2 sensitivity comes from its being on the border line of genetics and virus studies. It seems that its complete understanding, including the working out of the chemical chain of reactions which links the genoid to its phenotypic effect, and of the conditions of its multiplication inside and perhaps outside the *Drosophila* cells, would throw a valuable light upon the relations between genes and viruses and the activities of both. Such a complete understanding is perhaps not out of reach, since many and diverse lines of investigation may converge upon it ; but it will need the co-operation of insect physiologists, biochemists and geneticists. This makes the writer the more anxious for every valuable suggestion which will help him and his colleagues.

The present paper is intended to give a complete account of the present state of the problem, taking into consideration published, as well as unpublished, experimental evidence. The investigations are still incomplete on many points, but a whole team is now working on the problem.

2. PHYSIOLOGY OF SENSITIVITY

(*i*) *The conditions for the poisoning effect of* CO_2

The poisoning effect of CO_2 on sensitive flies depends on duration of exposure, temperature and concentration.

Duration of exposure to the gas is not important at usual temperatures. After a very short exposure, the sensitive flies may display only a weak form of intoxication, *i.e.* they are capable of active, though unco-ordinated and convulsive movements and they may even, after some hours, recover. Lengthening the exposure, the other conditions being kept constant, leads at first to less active movements and to a more delayed and less certain recovery ; then a state of saturation is reached and nothing is gained by a further increase. The minimum duration to get the full effect of the gas is very short, about 15 seconds, when the CO_2 concentration is high enough. It increases somewhat when the latter is near the threshold value, soon to be described, but under no circumstances does it exceed a few minutes. In all the work on the influence of the concentration, a 5 minutes' exposure was always used as a standard ; several hours of exposure do not produce any more important effect, when enough time is allowed for the wakening up from narcosis.

From very recent work, it appears that duration of exposure plays a far more important rôle at low temperatures, *i.e.* in the region of the left branch of fig. 1. This branch might, consequently, have a different meaning from that of the other.

When at a given temperature sensitive flies are exposed to atmospheres containing various concentrations of CO_2, the following facts are observed. Too low a concentration produces neither narcosis nor intoxication ; then, increasing the concentration, one comes first to a *narcosis threshold*, which seems about the same with sensitive and resistant flies. At first, both types of flies wake up in the same way, when they are returned to a normal atmosphere. Further on there occurs for the sensitive flies a *toxicity threshold*, above which they are injured by the treatment. As the concentration rises above the threshold, the amount of poisoning obtained increases, at first quickly, then very slowly, towards the limit which is reached with a pure CO_2 treatment.

There is some variation of this threshold value according to the flies used. Apart from an important random individual variation, many factors, such as the age of the flies, their genetic origin, the temperature at which they have been raised and kept, and perhaps many other still unsuspected agents seem to be operative. The data used in the drawing of fig. 1 are more or less average values. They refer to the concentration which, at each temperature, is able to poison about 50 per cent. of flies of the original sensitive stock, a few days old at the time of the test.

The temperature at which the test is made exercises a preponderant influence on the threshold value. As shown by fig. 1, there is a minimum at about $+2°$, on either side of which the curve rises steeply in a regular linear way. Above $+23°$ the CO_2 does not exercise any poisoning effect on sensitive flies, at the usual pressure of one atmosphere. Dr Kalmus tells me he has some evidence that the curve can be extended beyond this temperature by using higher pressures.

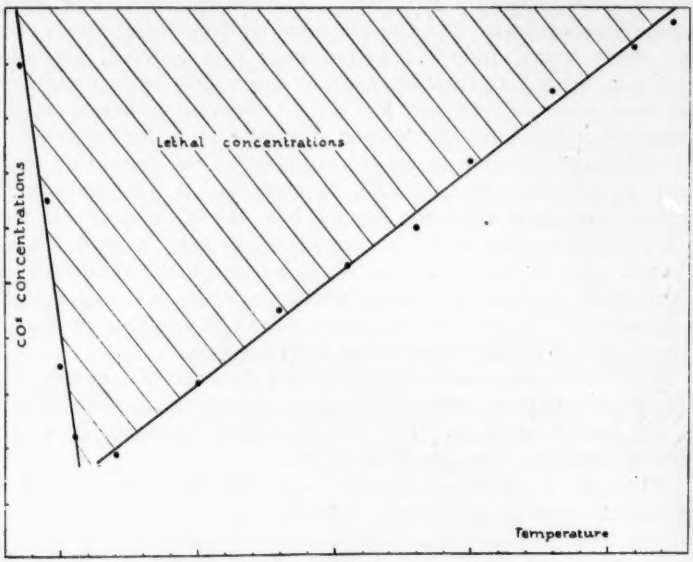

FIG. 1.—Toxic action of CO_2 on sensitive *Drosophila*.

The threshold CO_2 concentration does not depend upon the nature of the gas which is mixed with CO_2. This may be air, oxygen, nitrogen or hydrogen, without altering in any way the phenomena. The only relevant factor seems to be the partial CO_2 pressure.

(*ii*) *Adaptation of sensitive flies to lethal concentrations*

Striking adaptations are observed, when at the same temperature sensitive flies are subjected to a lethal CO_2 concentration soon after an exposure to a concentration below the threshold (subliminal concentration). The point needs still more careful investigation, but the present evidence can be summed up as follows.

Any subliminal treatment has the effect of enabling the flies to resist exposure to pure CO_2 for some time. This physiological adaptation lasts only a short time, depending chiefly upon the duration of the subliminal treatment and secondarily upon the

concentration used. When the exposure to the subliminal concentration is increased from half a minute to several hours, the adaptation time starts from a few seconds and rises more and more slowly up to about 15 or 20 minutes. It depends very little, if at all, upon the nature of the atmosphere in which the flies are kept when taken out of the subliminal concentration. For instance, when 5 minutes is the measured duration of adaptation the adapted sensitive flies, at their emergence from the adaptational treatment, can sustain equally well an exposure of 5 minutes to pure CO_2 and a 4 minutes' exposure to air or some other gas, followed by one minute of treatment by pure CO_2.

When adapted flies are submitted to some lethal supraliminal concentration, but not to pure CO_2, the result of the adaptational process is not only to make this concentration innocuous but indeed to render it itself adaptational. In some instances a 5 minutes' adaptational treatment enabled sensitive flies to remain for hours, without showing any poisoning, in a concentration which would have killed them in a few seconds if it had been used first. At the end of the experiment such flies were able to resist for some time exposure to pure CO_2.

Adaptation bears no relation to narcosis, as non-narcotic subliminal concentrations may be adaptational, and adaptation usually lasts longer than the narcosis produced by the adaptational treatment. Besides, flies can be narcotised by many agents other than CO_2, for instance by inert gases such as nitrogen or hydrogen, by poisons such as carbon monoxide or cyanic acid, or by anæsthetics like ether. No such narcosis seems to produce any major effect in the reaction of sensitive flies to a lethal CO_2 concentration. CO_2, therefore, seems to be as specific in its adaptational as in its toxic action.

The biochemical mechanism through which such an adaptation effect can be brought about is of course at present as unknown as the mechanism of the toxic effect itself. One may tentatively assume that some chemical precursor, the presence of which is needed for the toxic process, might combine with CO_2, when at subliminal concentrations, to give an innocuous compound. This reaction might be reversible allowing the precursor to be freed, after a time, for the toxic effect.

(*iii*) *Other physiological investigations*

A great deal of work has been done to get an insight into the physiological mechanism of the CO_2 intoxication of the sensitive flies. It had to be performed in a somewhat unsystematic way, as not much guidance was found in investigating such a puzzling phenomenon.

First, we tried to find out whether sensitive flies exhibit any other physiological difference when compared to resistant ones. The answer was always negative. A sensitive stock does not appear in any way weak or diseased; its fertility and general viability are

excellent. Treatment by many chemicals such as N_2, H_2, CO, HCN, H_2S, $H—CO_2H$, $CO_2H—CO_2H$, HCl failed to show any major discrepancy. Of course minor discrepancies are to be expected between stocks of different genetic origins, but none appeared to be linked with the CO_2 sensitivity. Similarly, no chemical introduced into sensitive flies, either through injection or through the mouth, has been found to change their behaviour with respect to CO_2. However, so few chemicals have been tried in this way among the many which might be suspected, a priori, to be active, that not much stress must be put on this negative result.

Then it has been shown that the CO_2 sensitivity has nothing to do with stigmata or respiratory movements. These are localised in the abdomen, and indeed sensitive flies, bereft of their abdomens, or even an isolated thorax, behave in precisely the same way as entire flies when treated with CO_2. Sensitivity is only made apparent in the movements of the legs and wings, so that there seems to be no way of showing it in isolated heads or abdomens.

Impressive, though perhaps not entirely convincing evidence has been collected, showing that the sensitivity is not due to a special property of the blood but rather to a reaction taking place at the cellular level.

(i) Resistant flies were injected with a large amount of hæmolymph drawn from sensitives. Treated by CO_2, either immediately or a few hours afterwards, they never showed any symptom of even a weak sensitivity. As will be said farther on, such flies do actually become sensitive. But, as this takes place 10 or 15 days after the injection, it is interpreted as the outcome of the invasion of the organism by the genoids carried by the injected hæmolymph. It seems that a far more immediate result would have been expected had the sensitivity been related to some property of the blood.

(ii) Moreover, injection into resistant flies of hæmolymph from CO_2-poisoned sensitives brings out a similar argument. Such flies are not affected by the injection. They should show some kind of poisoning had the hæmolymph of sensitives acquired some poisoning properties following exposure to CO_2.

(iii) Finally and conversely the CO_2 behaviour of sensitives is not changed by injecting into them some hæmolymph from resistants. The same negative result is obtained when the fly is "washed" with Ringer. This washing is made in driving a large amount of Ringer into the abdomen and letting it pour out of a hole made in the head. This drastic treatment does not injure the fly much and does not make it any less sensitive.

It seems likely that the toxic effect of CO_2 is localised in the thoracic nervous ganglion. The convulsive movements of the intoxicated flies point of course to a nervous injury, and the thoracic centre must be damaged since poisoned flies do not behave at all like decapitated flies and since the latter clearly manifest sensitivity.

Moreover, some tests were made which indicate that the thoracic ganglion is the only nervous centre to be injured, the cerebral ganglia remaining unhurt. The head of a sensitive fly was introduced into a small hole bored through an extended india rubber membrane. When the membrane had been left to contract, the fly head was perfectly isolated from the rest of the body and could be exposed alone to CO_2. The fact that the head bears no stigmata does not prevent, it seems, the gas reaching the cerebral ganglia by diffusion, since, when the head and the thorax are similarly isolated from the abdomen and exposed to CO_2, the fly shows both narcosis and poisoning in the normal way. But to make the access of the gas inside the head easier, one eye of the fly was removed in some experiments. Neither narcosis nor poisoning was ever observed in these experiments, which involved only a cephalic treatment. When freed the flies moved and fed normally and could be kept any length of time. The evidence seems then strongly in favour of the view that it is the motor nervous system alone, localised in the thorax, which is injured by CO_2.

(iv) CO_2 sensitivity in larvæ, eggs and pupæ

Most of the work on the physiology of the CO_2 sensitivity has been done with imagos, on which sensitivity is easiest to demonstrate. But larvæ from a sensitive stock are injured also by an exposure to CO_2 under temperature and pressure conditions which are, approximatively at least, the same as when imagos are dealt with. As the movements of the maggots are less elaborate and active than those of the flies, their state of poisoning may not be clearly apparent. When they wake up from the narcosis they may creep about, try to feed and even succeed in undergoing the pupal metamorphosis. But usually they die either as larvæ or as pupæ and do not reach the adult stage. A few of them, however, achieve an entire recovery and become flies. These show the normal sensitive behaviour, the larval treatment having produced no curative effect.

It has not been possible to demonstrate any sensitivity in the eggs or pupæ of sensitive stocks. Like those from resistant stocks, these dormant stages may remain in pure CO_2 for hours without any injury.

3. INHERITANCE OF SENSITIVITY

(i) Empirical rules

The original sensitive stock, as well as many others which have been obtained by different methods, breeds nearly true for sensitivity, and usually can be kept in mass cultures through many generations without requiring any selection. Nevertheless, even at the optimal temperature, *i.e.* 20°, a few resistant flies appear in the cultures from time to time. They behave, genetically, as individuals of pure resistant stock. When such a fly happens to be picked up among

the female parents, the ensuing generation is found to be a mixture of sensitives and resistants. By selecting sensitive female parents for one or two generations, the original, approximately true breeding, condition is easily restored.

The cross of two resistant flies, whatever their genetic origins, leads to an entirely resistant progeny. This statement is universal; but it must be noticed that it refers only to permanently resistant individuals: flies made temporarily resistant by a temperature cure may have sensitive offspring.

When a female from a sensitive stock is crossed with a resistant male, the whole progeny is sensitive and behaves genetically like individuals from the pure sensitive stock. Recurrent backcrosses to resistant males may lead, for many generations, only to sensitive offspring. Sometimes, however, some resistants appear here and there, but it is not certain that these " mutations " are actually more frequent in such a sequence of backcrosses than within a pure stock, as stated earlier. It may then be stated, as a fair approximation, that sensitivity is regularly carried along without segregation through a line of females.

The constancy of the transmission of sensitivity by females is probably under control of the genotype. Indeed, sensitive stocks of different genotypes differ in their abilities to breed true for sensitivity. But a given stock also varies from time to time with respect to this ability, probably as a result of the fortuitous selection at some generation of individuals poor in genoids. Thus, one has to be careful in attributing to the genotype a difference which may happen to exist between two stocks at a certain time.

The reverse cross of a resistant female to a male from a sensitive stock leads always to a mixture of sensitive and resistant offspring. Among the sensitives, males are only somatically sensitive and in no case transmit the character, whereas females produce a variable mixture of both types of offspring. Starting from such an initial cross it is possible, though sometimes difficult, to restore a good true breeding stock by selecting sensitive females for a few generations. Sensitivity may be also carried along mixed with resistance through an indefinite number of generations, by an alternate sequence of sensitive males and females, the other partner of the cross being resistant.

(*ii*) *Factors controlling the transmission of sensitivity by males*

The ratio of sensitives among the offspring of a sensitive male crossed with a resistant female varies from 0 to about 90 per cent., but never reaches 100 per cent. It is controlled by many factors, which are not yet entirely understood and are very intricate.

Apart from the action of the temperature on deposited eggs and on the male before copulation, each individual male exhibits a certain intrinsic potentiality to produce a high or low ratio of

" infected " spermatozoa. Part of this variation is apparently random and irreducible, the rest seems inherited. Now, each parent hands down to each male offspring two things—a haploid nucleus or genome and a certain amount of genoids. The genoids inherited from the male parent do not seem to influence the ability of the sons to carry sensitivity in their germ cells. This statement generalises the fact, already pointed out, that a male never transmits sensitivity when it has inherited it only from its father.

In contrast to the sperm, the amount and distribution of genoids within the egg, prior to fertilisation, are certainly important. They may be dependent on the genetic origin of the mother and on the circumstances of its life. Thus the problem is highly complex ; in this peculiar field of investigations no sharp line can be drawn between environmental and genetic factors since, as will be seen farther on, acquired effects are distinctly inherited.

There is some evidence, better than in the case of the females, that the chromosomal genotype may control the transmission of sensitivity by males. Among the stocks which were made sensitive, some were found in which the males transmit the character to only a very small part of their progeny, and even some in which they do not transmit it at all. Within such stocks the inheritance of sensitivity is purely maternal. This influence of the genes is indirect and delayed. The genotype of the reproducing male itself has been demonstrated to exert some action ; the genotype of its mother is active, but the genotypes of former maternal ancestors must also be taken into consideration. When genoids are introduced into a stock of a certain genotype it looks as if, after a few generations, some kind of equilibrium is reached which determines the average content in genoids of both male and female gametes. Any crossing with another stock upsets this equilibrium. As the transmission of the genoids is only remotely connected with the genes, it is not easy to recognise individual actions of genes during the genetic segregation which follows the cross. Until a gene is found which exerts a major influence on the phenomenon, careful and lengthy experiments must be performed to reveal such minor genic actions. Investigations of that kind are being actively pursued.

Another factor which plays a distinct rôle in the transmission of sensitivity by the males is their age. When a set of sensitive males is kept copulating with a fresh batch of resistant females daily, the proportion of sensitives among the progenies at first decreases rather sharply, then this decrease slows down and, finally, an actual slight increase occurs towards the end of the reproductive period of the males, *i.e.* 15 or 20 days. This is but an average course ; there is a large variation between individual males.

A similar decrease in the proportions of sensitives is observed when two identical sets of males are mated with similar resistant females, the first set being mated as soon as they reach the adult stage, the second after a period of ageing, during which they are kept virgin.

Finally, there is also a decrease in the proportion of sensitives among the eggs laid successively, after one copulation with a sensitive male by resistant females.

The last fact seems to imply either that the infected spermatozoa have a greater death-rate than the others, or that the genoids disappear spontaneously within them. The same process, whatever it may be, must occur in the males and account for part of the variation in the proportion of sensitives among their successive progenies; but the phenomena are made more complex in this case by the fact that spermatogenesis being a more or less continuous process, the age of the successive batches of spermatozoa does not correspond necessarily with the age of the male. The final slight increase of sensitives, referred to above, might be accounted for on this ground. The last spermatozoa used by a male are supplied by the imaginal spermatogenesis which has been shown to take place in *Drosophila* (Philip, 1942), and hence these spermatozoa may not be very old at the time of fertilisation.

Finally, the last factor which has been investigated is the genotype of the resistant females used in the cross. When identical sets of sensitive males are crossed with females of different genotypes, significant though rather slight differences in the proportions of sensitives may be observed among the different progenies. It does not seem worth while to review the several hypotheses which might account for this fact, as they must remain at present so highly speculative.

(*iii*) *Mechanism of transmission of sensitivity by males*

Sensitivity might be supposed to be transmitted by males, a priori, in two ways—genoids may be included within the spermatozoon, as was assumed above, or they may be present only in the spermatic liquid and may be carried into the egg mechanically at the time of fertilisation. Some evidence to decide which hypothesis is true may be derived from polyandric fertilisation. When a resistant female is crossed simultaneously with two males, one resistant and the other sensitive, a few exceptional sensitives, about 5 per cent., are found among the progeny of the resistant father. The offspring of the other male show a variable but always much higher proportion of sensitives, the same indeed as a control cross involving similar resistant females and sensitive males alone. Polyandric females were demonstrated to give birth, day after day, to a thorough mixture of both kinds of offspring; the two samples of sperm must therefore blend in their seminal receptacles. If the genoids were present in the spermatic liquid and not within the spermatozoa, such a difference in the proportions of sensitives should not be observed between the progenies of the two kinds of males. The facts are consistent, therefore, with the assumption that the spermatozoa themselves include the genoids.

If such is the case, the appearance of some sensitives among the progeny of the resistant males must be accounted for by polyspermic fertilisation which is usual in *Drosophila* (Huettner, 1924). There is no difficulty in assuming that a genoid, introduced into an egg by a

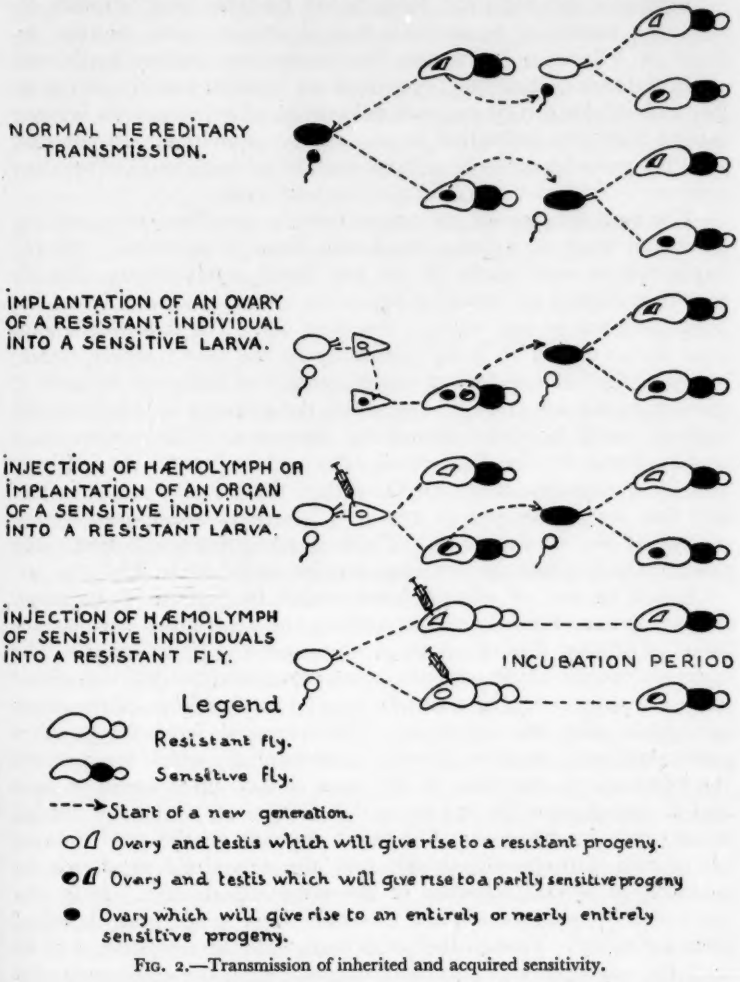
NORMAL HEREDITARY TRANSMISSION.
IMPLANTATION OF AN OVARY OF A RESISTANT INDIVIDUAL INTO A SENSITIVE LARVA.
INJECTION OF HÆMOLYMPH OR IMPLANTATION OF AN ORGAN OF A SENSITIVE INDIVIDUAL INTO A RESISTANT LARVA.
INJECTION OF HÆMOLYMPH OF SENSITIVE INDIVIDUALS INTO A RESISTANT FLY.
INCUBATION PERIOD
Legend
Resistant fly.
Sensitive fly.
Start of a new generation.
Ovary and testis which will give rise to a resistant progeny.
Ovary and testis which will give rise to a partly sensitive progeny
Ovary which will give rise to an entirely or nearly entirely sensitive progeny.

FIG. 2.—Transmission of inherited and acquired sensitivity.

supernumerary spermatozoon, may succeed in invading the organism. Now although most of the eggs must be penetrated by both kinds of spermatozoa, there are very few exceptional sensitive flies. Genoids in supernumerary spermatozoa must, therefore, have only a small chance of establishing themselves in the egg. It may be that they are not brought to the right place inside the egg.

4. TRANSMISSION OF SENSITIVITY BY ORGAN IMPLANTATION AND INJECTION

(*i*) *Somatic acquisition of sensitivity*

Extensive investigations have failed to show any resistant fly acquiring sensitivity by mere biological contact with sensitive individuals. Resistant larvæ may live in the same culture bottle with sensitive larvæ ; they may even feed on crushed sensitive larvæ or flies and still keep their resistant behaviour. Also, sensitivity is never carried from one individual to another by copulation. In contrast, artificial procedures, such as implantation or injection, convert any resistant individual very easily into a sensitive one.

The implantation of an organ from a sensitive larva into an individual from a resistant stock can make it sensitive. All the implantations were made at the last larval instar stage. The fly may then display the sensitive behaviour either as soon as it hatches from the pupa or only when a few days old. The following organs were demonstrated to bring sensitivity to the host : ovary, brain, imaginal discs of eye, legs or wings, portion of the gut. In most of the individuals which became sensitive, the presence of the developed implant could be demonstrated by dissection, while nothing was usually found in the flies which remained resistant. As implants may be overlooked, it seems likely that the correlation is absolute and that the appearance of sensitivity is strictly conditioned by the success of the implantation. There is no difference between the readiness with which the two sexes acquire sensitivity in this way.

Injections are as effective and easier to perform than organ implantations. Hæmolymph as well as the centrifuged supernatant liquid of crushed flies of any stage were shown to be infectious. The injection can be made with the same permanent success into either larvæ or imagos. It takes a certain time for the symptoms of sensitivity to appear after the injection. This so-called *incubation period* is particularly easy to show, when it is the adult fly which has received the injection. It may last at 20° from 10 days up to about 30 days and is not changed by the successive CO_2 tests, which are needed to ascertain its duration. The latter depends on the total amount of genoids introduced initially into the organism, as shown by injection of several dilutions of the same supernatant. It is also controlled by temperature, and increases when temperature is raised from 20° to 25°. Though this point needs fuller investigation, it looks as if the appearance of sensitivity depends upon the attainment of a certain level in the invasion of the organism by the genoids. The time needed to reach this threshold must be of course the shorter, the higher the initial level of infection and therefore the lower the dilution of the infectious liquid.

The infectiousness of a liquid, whether diluted or not, is apt to disappear in a few hours when it is kept at room temperature. At

higher temperatures this inactivation becomes more rapid and must be nearly instantaneous at 60°. Cooling, on the contrary, helps to preserve the activity. Some infectious liquids were kept two days at −7° without entirely losing their activity. In ordinary laboratory routine, the liquids used for the injections are always kept at about 0° and all the necessary centrifugations are made in a cooled room.

We have tried to ascertain the size of the infective particles, using ultrafiltration on collodion membranes. Filtration through a 180 mμ membrane has been found to preserve some of the activity of an infectious liquid. Activity is however much depressed by the process, making it likely that the 180 mμ membrane is not far from the limit below which no infective particle will be found in the filtrate. This still crude measurement will be completed later on ; it already shows that the size of the genoid in *Drosophila* extracts lies well within the usual range of virus particles.

(II) *Inheritance of the acquired sensitivity*

The most striking aspect of the acquisition of sensitivity by implantation or injection is the ability of the genoids to penetrate inside the germ cells and be carried by the gametes to the next generation. The operation has indeed the unusual consequence of changing not only the physiology of the individual but also that of its progeny, for a potentially unlimited number of generations. The chief features of this phenomenon are schematically summarised in fig. 2, which shows, for comparison's sake, the rules of the normal hereditary transmission of sensitivity as well.

The two sexes, as will be seen, display very different abilities to become germinally infected, and this contrasts sharply with the equal readiness with which they become somatically sensitive. A male never transmits sensitivity, unless it is the offspring of a female in whose germ cells the genoids were already present at an early stage of oögenesis. This early infection is possible in only two cases : first when the mother is sensitive congenitally, *i.e.* has inherited sensitivity ; secondly, when a resistant ovary is grafted into a sensitive foster-mother. Acquired sensitivity then is not inherited in the males, even when this acquisition reaches back to the time of the fertilisation, *i.e.* when the genoid is brought into the egg by the spermatozoon.

A female, on the contrary, in whatever way she has acquired sensitivity, may always produce at least a few sensitive offspring. This is observed even when the somatic infection takes place at so late a stage as the imaginal one. But there is a large variation of the proportions of sensitives among such progenies and the 100 or near 100 per cent point, which is characteristic of females of pure sensitive stock, is reached only in about the same circumstances as those which allow the males to transmit the sensitivity.

To sum up, there appear to be two situations with respect to transmission by the gametes :—

(i) The ovum is already infected at an early stage of the oögenesis. In this case, if the ovum develops into a female, it will have 100 per cent sensitive progeny or nearly so ; if it develops into a male, it will transmit the sensitivity to some of its offspring.

(ii) The infection takes place too late, *i.e.* either during the individual's life, or too short a time before conception. A male will then produce only resistant offspring and a female a mixture of sensitives and resistants.

The ultimate difference between the two cases might well rest upon the inclusion of some genoids in the primordial germ cells at the time of their formation. This might happen only when the ovum is infected long before fertilisation, *i.e.* in the first case. In the second case, a secondary infection of the gonads would succeed, in the females, in compensating partly for the lack of genoids in these primordial germ cells ; but, in the males, such a secondary infection would not take place for an unknown reason. Anyway, it is worth noticing that when the genoids are artificially introduced into a resistant stock they can utilise only one door to enter the germ cells, *i.e.* the oögonial cells. Afterwards, in the later generations, they are of course present in the spermatogonial cells as well. It remains unknown whether the genoids can penetrate the oögonial cells at a special stage only or at any time during the oögenesis.

All that has been said about the inheritance of acquired sensitivity refers to *Drosophila melanogaster*. Inside this species, no evidence was found of a control by the chromosomic genome of the fundamental facts set forth here. Three other species, *D. simulans*, *D. funebris* and *Calliphora vomitoria*, were tested for their abilities to acquire sensitivity. The last seems to be immune, but both *Drosophila* species acquire it very readily. An outstanding difference, however, is that the character is inherited in *D. simulans*, as it is in *D. melanogaster*, but there is no evidence thus far of its inheritance in *D. funebris*. Thus, if the gametes of this species can carry the genoid at all, it must be either a very rare event or must happen in circumstances still to be determined.

5. TEMPERATURE CURES OF SENSITIVITY

Temperatures above 30° are able to cure the sensitivity either temporarily or permanently, according to the genetic origin of the flies and their developmental stage at the time of treatment. A further effect of high temperature is to prevent the regular hereditary transmission of the sensitivity : it cures the germ cells.

(i) *Temporary cures*

Any sensitive fly, whatever its genetic origin and however it has acquired sensitivity, may be induced very easily to assume for a

time a resistant behaviour by heating it above 30° during either the pupal or the imaginal stage. When the fly is returned to 20° sensitivity reappears in a few days. The transitional stages between sensitivity and resistance are difficult to study, for the organism goes through them quickly either forwards or backwards. It appears from preliminary work that for " half-cured " flies the temperature-CO_2 concentration curve shifts both upwards and towards the right in relation to the curve characteristic of normal control flies, so that its minimum is at a higher temperature. This displacement goes on until the whole curve vanishes ; then the flies appear to be resistant whatever the temperature and the CO_2 concentration.

The duration of heat treatment required to produce a complete temporary cure depends on the temperature and on the developmental stage at which it is applied. At 34°, about the highest temperature which can be used without injuring the flies, one day and a half is enough to cure young imagos. With lower temperatures this duration increases at first slowly, then very quickly in the neighbourhood of 30°. Old flies are somewhat more difficult to cure than young ones.

When the heating takes place during the pupal period 24 hours are sufficient, even at 30°, provided they are at the end of this stage. But such a short treatment, even at a higher temperature, has no effect when it takes place at the beginning of pupation. This does not prove, however, that the temperature is more effective on the last pupal day. The same process, which brings back sensitivity in the imago when the heating ceases, may indeed operate in the pupa, where it is bound to be overlooked as there is no way of showing sensitivity in a pupa.

A very strange, as yet utterly unaccountable feature of the temperature cures can be observed when sensitive flies are subjected to two heat treatments, the first one during the embryonic or the larval stage, the second during the pupal stage. It is found that the last one, which should have cured the flies very easily, had it occurred alone, is made less effective by the first. This *protection effect* (it is a protection against the cure) can be observed only with flies, the sensitivity of which comes from the mother ; with flies having inherited sensitivity from their father alone, the embryonic or larval treatment produces a permanent cure.

Finally, a last fact about the temporary cures was secured by injection experiments. It was found that the centrifuged extract from temporarily cured flies is not infectious. Since the flies can recover sensitivity spontaneously, some genoids must of course be stored somewhere in the organism. One is led to assume that they are either rare or in such a location that the grinding of the flies does not allow them to escape in an active condition in the supernatant.

(*ii*) *Permanent cures*

When the progeny from the cross of a resistant female by a sensitivity-transmitting male is heat treated, during either the embryonic or the larval stage, it is found to include fewer sensitives than if no heat treatment had been used, or none at all. This non-occurrence of the normally expected sensitive offspring is the result of an actual cure and not of a higher mortality of the sensitives. This cure is permanent : sensitivity has never been observed to recur in any such individuals.

The effectiveness of a heat treatment depends on the temperature and even more on the stage of development. The most susceptible stage is the beginning of embryonic development. Heating the eggs for 12 hours at 32°, as soon as they are laid, suffices to cure most or even all of the individuals. Heating the larvæ is far less effective, and the whole larval period, *i.e.* about 4 days, must be subjected to the treatment to get a complete cure. The susceptibility to the cure disappears entirely with pupal metamorphosis ; after this event, as we saw, only temporary cure can be produced.

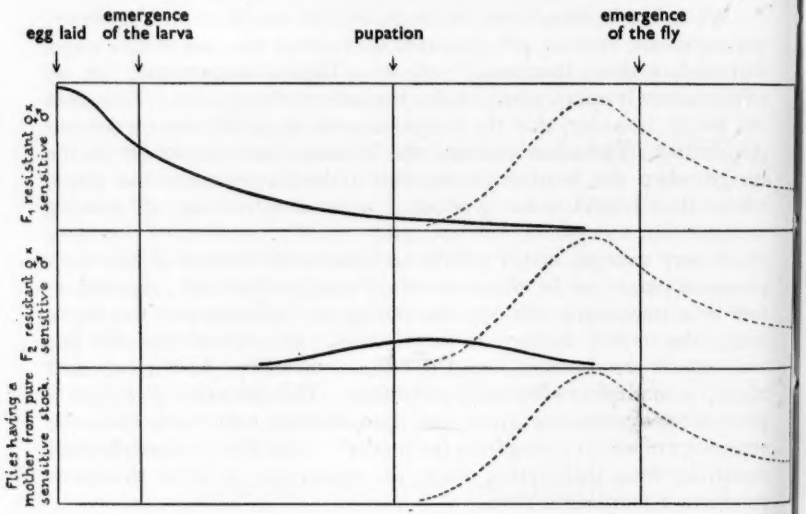
egg laid
emergence of the larva
pupation
emergence of the fly
F1 resistant ♀ × sensitive ♂
F2 resistant ♀ × sensitive ♂
Flies having a mother from pure sensitive stock.

FIG. 3.—Responsiveness of sensitive flies of different genetic origins to short heat treatments at different stages of development. Full line : permanent cure. Broken line : temporary cure.

It is very striking to compare the effect of heating the embryonic or larval stages of flies, which have inherited sensitivity from their father alone, with its effect on flies from a sensitive stock. With these there is no permanent cure but only the protection effect, which means rather what could be called an exaggeration of sensitivity. A kind of behaviour intermediate between that of the two kinds of

sensitive flies is shown by the second generation from the cross of a resistant female with a sensitive male, which includes, as we saw, both types—sensitives and resistants. When such progeny is subjected to heat treatments at different stages the embryonic treatment produces merely a protection effect, while larval treatment gives some permanent cures. Temporary cure is obtained of course in the usual circumstances.

The rather intricate features of these somatic temperature cures are summarised in fig. 3, where the ordinates are arbitrary scales of units of what can be called the responsiveness to the heat treatment.

(*iii*) *Gametic cures*

Investigations are at present being made on gametic cures and it is hoped that the details will soon be worked out.

From preliminary work it appears that any heat treatment of the male from the laying of the egg up to the imaginal metamorphosis lessens the proportion of sensitives among its progeny. But the ability of mature spermatozoa to carry sensitivity is not changed by the temperature, as is shown by submitting adult males or fertilised females to heat treatments. The period of responsiveness to temperature therefore covers the period from the isolation of the primordial germ cells to the completion of the spermatozoon, but does not extend to the life of the sperm itself.

Heat treated females, similarly, are found to give a smaller proportion of sensitives among their offspring, even when it is the egg-laying fly which is heated. The temperature is thus also active during the whole period of development of the ovum.

When a partial or complete gametic cure happens to coincide with a somatic cure, this may be looked upon as a case of inheritance of an acquired character. It occurs regularly with permanently cured flies which never breed any sensitive offspring. The temporary cure may or may not be inherited, according to the duration of the heat treatment and stage in the life cycle at which it is applied. On the other hand, it is known from transplantation experiments that in females the germ cells can acquire the genoids from the somatic cells and *vice versa*. In my opinion these acquired character inheritances have no deep biological significance, in the sense that they do not involve any direct and constant action of the somatic cells on the germ cells. The inheritance of the acquired cure seems to be rather the result of the identity of responses of both kinds of cell to the same external condition, the temperature. The reciprocal infection of somatic and germ cells does not seem to agree any better with the progressive germinal response to somatic adaptative changes, which is assumed to take place by Lamarckian-minded biologists.

(iv) *Mechanism of the temperature cures*

We cannot yet give a definitive account of the mechanism of temperature action, and to work out a plausible hypothesis is only worth while to the extent it may prove to be an incentive to new experiments.

First, it must be noticed that similar phenomena are to be found in Sonneborn's observations (1946, 1947) on "killer" varieties of *Paramecium*. When "killer" *Paramecia* are exposed to a temperature of 38·5° they turn into sensitives. With a long enough exposure a pure sensitive progeny can be grown from them; but with short exposures part of the progeny at least reverts to killers. Sonneborn has accounted for these observations in a satisfactory way, on the basis that high temperature destroys at a fairly low rate the cytoplasmic particles ("kappa" particles) which are held responsible for the killer behaviour. Short exposures allow some particles to remain uninjured, but they are too few for the *Paramecium* to be a killer, as this needs a high kappa concentration. Later, the multiplication of kappa outpaces the cellular division rate, hence the reversion to the killer behaviour. The outpacing is greatly favoured by autogamy.

Both permanent and temporary cures have also been observed with some plant virus diseases, consequent on to heat treatments. In some cases heating the infected plants leads only to a masking of the symptoms of the disease, which reappear when the plants have been returned to a normal temperature. In other cases a true permanent cure is obtained. In the writer's knowledge no thorough study of these cases has been made (Johnson, 1922; Tompkins, 1926; Bennett, 1927; Crainger, 1936; Kunkel, 1936; Martin, 1930).

In the writer's opinion there is a close analogy between the hereditary CO_2 sensitivity in *Drosophila* and the killer case in *Paramecium*, and Sonneborn's kappa particle and the CO_2 genoid are likely to be very similar biological objects. Nevertheless, the temperature action is more complex in *Drosophila*, in relation it seems to the metazoan condition. With *Paramecium* the permanent cure is the extreme stage of the temporary cure; but with CO_2 sensitivity the two cures are never the result of the same treatments. One must then try to account separately for the two cases, although of course they may be related in some obscure unknown way.

Since the temporary cure always takes place in the same circumstances, whatever the genetic origin of the fly, it must be independant of the amount of genoids present in the egg. Now this initial amount is known to control the infection of the germ cells, but may not have any influence on the ultimate somatic concentration and location of the genoids inside the late pupa or adult fly. If such is the case it is not surprising that a heat treatment, which is applied to these late stages, brings out a uniform response. It was formerly assumed

(L'Héritier and Sigot, 1946) that the temperature destroys some intermediate substance in the chain of reactions, which is supposed to link the genoids with their physiological effect. The heated fly was therefore bound to appear resistant, until the substance has returned to its normal concentration. However, it was recently found that the centrifuged supernatant of temporarily cured flies is no longer infectious. Heating must then damage the genoids themselves and not some alleged product of their activity. Some parts of the organism must of course remain infected to allow the reversion to sensitivity, so that the resistance of the genoids to temperature probably varies with their location.

Coming now to the permanent cure, it seems significant that to produce this effect a heat treatment must be applied during the growth of the organism and to individuals whose initial condition of infection is low. This suggests a correlation with cellular multiplication which is found also in the gametic cures, since ripe gametes are not influenced by heat which is active only during gametogenesis. It was believed at first (L'Héritier and Sigot, *l.c.*) that the temperature acted in inducing a discrepancy between the rates of multiplication of the genoids and of the cells. At high temperatures the cells would outpace the genoids and thus be freed of them. For this process to succeed in freeing the whole organism, the genoids should not be too numerous at the beginning of development, which means that they should have been carried only by the spermatozoon. It is not surprising that gametic cures are not similarly restricted, since gametogenesis involves so many cell divisions, which is bound to make the outpacing more effective than during somatic development. This hypothesis is very similar to Sonneborn's so-called concentration hypothesis, confirmed by Preer (1946), who found that "killer" *Paramecia* lose the killer character when they are cultivated in a rich and frequently renewed medium. Such conditions allow a rapid sequence of divisions and the kappa particles are not able to keep pace.

To account for the permanent cures of CO_2 sensitivity, this hypothesis is not however entirely satisfactory, since it does not make allowance for the protection effect. Although it may be understood why heating the embryonic and larval stages fails to free the whole organism from genoids, when they are very numerous in the egg, it should at least decrease their amount, and it looks like a paradox that this should make it more difficult to produce a temporary cure. Moreover, no direct evidence of such a decrease was found in injection experiments. When the infective capacity of the extract of larvæ from a pure sensitive stock, which have been heated at 30° through their embryonic and larval life, was compared with a control extract, no distinct decrease of infectiveness was found.

In view of this new evidence, the assumption of a discrepancy between the rates of multiplication of the cells and the genoids, brought

about by the raising of the temperature, cannot be retained, at least in its former crude form. In the writer's opinion some mechanism of this kind probably accounts for the gametic cures, but the somatic permanent cure of those individuals which have received sensitivity from their father alone must pertain to some more complicated mechanism. When the genoids have been brought by the spermatozoon they may be located in some special part of the organism, at least during the embryonic and larval stages, instead of being diffused uniformly, as they probably are in individuals from a pure sensitive stock. This special location might make them prone to be destroyed by heat, as they always are in the pupa and the adult fly, whatever the genetic origin of the individual. Further speculation must be postponed until these assumptions have been tested.

6. GENERAL DISCUSSION

Though much remains to be done to clear up the problem of CO_2 sensitivity, it does not seem likely that any new evidence will alter fundamentally its general outlook and will strip the biological object, which has been called a genoid, of its ambiguous character. The puzzling question to be discussed is indeed whether the CO_2 sensitivity is a virus disease or is a case of cytoplasmic inheritance.

Three features of the CO_2 sensitivity do not appear to fit its assimilation to a virus disease. First, the sensitive flies are not diseased in any of the ordinary meanings of the word. Their viability, longevity and fertility are entirely normal and, though it has been much looked for, no way has ever been found to detect the sensitivity without exposing the fly to CO_2. Moreover, unlike most of the virus diseases sensitivity is not contagious in the natural circumstances. To introduce the genoid from the outside into an organism, artificial surgical means must be used. Finally, the regular inclusion of the pathological agent in the gametes is a very unusual feature for a virus disease.

It must be conceded, however, that none of these three differences will resist any sharp scrutiny when they are taken individually. Several cases are known of plants harbouring a virus without showing any symptom of disease. The presence of the virus in these so-called virus carriers can be detected by introducing it into another organism, which may be performed by needle inoculation, grafting or by an insect vector (Johnson, 1925 ; Smith, 1929 and 1930 ; Salmon and Ware, 1928 ; Thrupp, 1927). The classical case seems to be that of the potato variety King Edward ; it has been shown that every plant of this variety harbours a concealed virus that causes a typical disease to develop in any other potato plant which a King Edward shoot happens to be grafted on (Salaman and Le Pelley, 1930). Moreover, the insects or ticks which play so great a rôle in the spreading of some plant and animal virus diseases, harbour the agent without apparently

being diseased in any way. In some cases at least it has been demonstrated that the virus multiplies inside its animal vector (see *System of Bacteriology*, vol. vii, Medical Research Council, London, p. 444). Some viruses, therefore, are able to invade the cells of an organism without altering its general physiology and morphology; of course any exceptional and highly specific effect will have every chance of escaping detection.

Coming now to discuss the non-contagious character of the sensitivity, it must be noticed that though some virus diseases are highly contagious many of them depend for their spreading upon an artificial introduction into each of their successive hosts, which in some cases may be effected only by a specific insect. It is commonly assumed that the plant viruses are unable to penetrate the external surfaces of the leaves and stems, unless there happens to be some wound, which may be only the breaking of a trichome. Once inside the plant they spread by diffusion from cell to cell. The genoid is able, similarly, to cross the walls of the cells, since starting from an implanted organ it can invade the whole organism and is found ultimately even inside the gametes. Its inability to cross the hard external chitinal body wall is not surprising, and that it also fails to cross the digestive tract wall may only mean that it is destroyed by the digestive juices. There does not seem, consequently, to be any fundamental difference between the genoid and a pathological virus on this ground.

Concerning the third point, it must be noticed that though most of the virus diseases are never inherited, several cases have been reported where a plant virus is transmitted by seed and even by pollen. The case of the mosaic of bean has been studied rather extensively. Both ovules and pollen harbour the virus and give rise to infected seedlings, in an inconstant and irregular way (Nelson, 1932; Reddick, 1931; Harrison, 1935). The same general situation holds for the insect vectors. As a rule, the progeny of an infectious individual does not carry the virus, but there are notable exceptions. The wood tick, *Dermatocentor venustus*, which is responsible for the inoculation of Rocky Mountain spotted fever, regularly transmits the virus to its offspring. The presence of the agent has been demonstrated in the eggs, which are heavily infected (see *System of Bacteriology*, *l.c.*).

There is therefore no final ground on which to reject the assumption that the CO_2 sensitivity might be after all merely the physiological symptom of a very strange virus disease. It might be thought even that sensitive *Drosophila* is the insect vector of some disease, the chief host of which remains to be discovered. On the other hand, there is good ground for looking at the case as one of cytoplasmic inheritance. The character is inherited according to precise and elaborate rules, which allow the outcome of every cross to be predicted with nearly the same accuracy as when Mendelian characters are dealt with. The genoid is carried along by gametes of both sexes in a similar way

to albino plastids in the cases of cytoplasmically inherited variegations in plants and, from this point of view, may be looked at as a cytoplasmic hereditary unit, together with plastids and Sonneborn's kappa particles. Such objects, however, are not supposed usually to be able to cross the cell walls and plastids apparently do not. But the writer has indirectly learned that Sonneborn recently succeeded in showing that *Paramecia* could acquire kappa particles by contagion, without the intimate cytoplasmic contact which takes place during conjugation. Now, the strict dependance of kappa particles upon the genes and the constancy with which many hereditary characters are controlled by such particles in *Paramecium* preclude, apparently, their assimilation to viruses. Should this discovery be confirmed, the ability to penetrate the organism from the outside could not be used as a criterion to separate cytoplasmic units and viruses.

The similarity between the genoid and the autonomous cytoplasmic factors of *Paramecium* seems to end when the relation to the genome is taken into consideration. These cytoplasmic factors have been found in the so-called B group of varieties of *Paramecium aurelia*, where they control several hereditary differences. Each of these is autonomous, in the sense that no gene is able to initiate its formation, but it can multiply only in the presence of a specific dominant gene and is quickly lost when, following a sexual process, the genotype becomes homozygous for the recessive allele. In the A group of varieties of the same species, however, the hereditary characters are controlled directly by the genes, and though there is some sort of temporary cytoplasmic inheritance the cytoplasmic constituents are not autonomous and their formation can be initiated by the genes (Kimball, 1947). In *Paramecium*, therefore, there is a close connection between the hereditary cytoplasmic factors and the genes, whereas no major influence of the genes on the CO_2 sensitivity has ever been demonstrated. The fact that sensitivity can be inoculated into several species of *Drosophila* and, in some of them at least, is afterwards regularly inherited, shows even that the genoid can accommodate itself to very different genomes. The writer, however, is not inclined to hold this difference as fundamental. Some minor influences of the genes have actually been found and the discovery of a gene, which would prevent entirely the multiplication of the genoid, is not a priori an unlikely event. Without altering much the general outlook of the problem, this discovery would bridge much of the difference from the *Paramecium* case.

Too little is known about the kind of cytoplasmic inheritance which is inferred from the non-infrequent dissimilarity between reciprocal hybrids in plants, for any parallelism to be drawn with the CO_2 sensitivity. Therefore, to sum up the discussion, the writer expresses his feeling that no advance will come from classifying the CO_2 genoid in any definite category, since its classification is doomed to remain so much a question of definition and personal feelings. It seems more opportune to rely on future work to disclose in the

same organism or in others more or less similar facts, which will make it less speculative than it is at present to trace the general relations between the biological objects, which are called genes, plasmagenes and viruses.

7. SUMMARY

Sensitive Drosophilas display a very peculiar physiological behaviour, when they are exposed to CO_2. A contact of a few seconds with this gas can poison them irreversibly, whereas ordinary resistant Drosophilas can remain a long time in pure CO_2 without any permanent injury. The thoracic nervous ganglion is very likely the seat of the intoxication.

Sensitivity is transmitted in crosses as a hereditary unit, but shows no linkage with any chromosome and appears to be bound to some discrete cytoplasmic agent, which has been called a genoid. A female of a pure sensitive stock, crossed with any male, produces only sensitive offspring. A sequence of recurrent backcrosses to resistant males leads similarly to sensitive offspring, with only a few accidental resistants. The cross of a male from a pure sensitive stock to a resistant female produces a mixture of sensitives and resistants. Among the former, the males are only sensitive somatically and never breed any sensitive offspring, whereas the females are able to transmit sensitivity to part of their progeny.

Sensitivity is not contagious by mere natural contact, but can be acquired easily by the implantation of an organ of a sensitive individual or the injection of hæmolymph or extract of sensitives. Acquired sensitivity is always inherited in females, but a male never transmits this character unless it arises from an ovum which has been invaded by some genoids at an early stage of oögenesis.

Keeping sensitive flies at temperatures above 30° leads to cures of the sensitivity. These cures may be either permanent or temporary, according to the genetic origin of the flies used and the developmental stage at which they are heated. Moreover, heating can interfere with the normal hereditary transmission of sensitivity.

The question whether CO_2 sensitivity must be looked upon as a virus disease or as a case of cytoplasmic inheritance is discussed.

REFERENCES

BENNETT, C. W. 1927.
Mich. State Coll. Agric. Exp. Sta., Bot. Sect. Tech. Bull. No. 80.

CRAINGER, J. 1936.
Nature 137, 31.

EPHRUSSI, B., AND BEADLE, G. W. 1936.
Am. Nat. 70, 218-225.

HARRISON, A. L. 1935.
N.Y. State Agr. Exp. Sta. Tech. Bull. No. 236.

HUETTNER, A. F. 1924.
J. Morph. 249-265.

JOHNSON, J. 1922.
Phytopath. 12, 438-440.

JOHNSON, J. 1925.
Wisc. Agric. Exp. Sta. Res. Bull. No. 63.

KIMBALL, R. F. 1947.
Genetics 32, 486-499.

KUNKEL, L. O. 1936.
Phytopath. 26, 809.

L'HÉRITIER, PH., AND TEISSIER, G. 1938.
C.R. Ac. Sc. 205, 1099.

L'HÉRITIER, PH., AND TEISSIER, G. 1939.
C.R. Ac. Sc. 206, 1193 and 1683.

L'HÉRITIER, PH., AND TEISSIER, G. 1945.
Pub. Lab. E.N.S., Paris, Biologie Fasc. I.

L'HÉRITIER, PH., AND SIGOT, A. 1946.
Bul. Biol. Fr. et Bel. 80, 171-227.

L'HÉRITIER, PH., AND HUGON DE SCOEUX, F. 1947.
Bul. Biol. Fr. et Bel. 81, 70-91.

MARTIN, J. P. 1930.
Hawaï Planters Rec. 34, 375.

NELSON, R. 1932.
Mich. State Coll. Agric. Exp. Sta. Tech. Bull. No. 118.

PHILIP, U. 1942.
Nature 149, 527-528.

PREER, J. R. 1946.
P.N.A.S. 32, 247-253.

REDDICK, D. 1931.
Deux. Cong. Intern. Pathol. comp. 363.

SALAMAN, R. N., AND LE PELLEY, R. H. 1930.
P.R.S., B, *106*, 140.

SALMON, E. S., AND WARE, W. M. 1928.
Ann. Appl. Biol. 15, No. 3.

SMITH, K. H. 1929.
Ann. Appl. Biol. 16, No. 1.

SONNEBORN, T. M. 1946.
Cold Spring Harbor Symp. Quant. Biol. 11, 236-255.

SONNEBORN, T. M. 1947.
Adv. in Genet. 1, 263-358.

TOMPKINS, C. M. 1926.
Phytopath. 16, 581-609.

THRUPP, T. C. 1927.
Ann. Appl. Biol. 14, 175.

INTRA-SEXUAL SELECTION IN *DROSOPHILA*

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INTRODUCTION

SINCE Darwin first wrote on the subject in 1871, sexual selection has been generally accepted as one of the basic facts of biology. The evidence in its favour seems, however, to be mainly circumstantial. Its existence has usually been inferred from sex differences depending on what are called secondary sexual characters which are supposed to have arisen as results of that selection. Such an approach has its dangers, and Huxley (1938) has made important criticisms of the original concept of sexual selection. He has shown that a large number of characters which have been attributed to sexual selection are unconnected with competition for mates. This is particularly the case in monogamous birds which offer some of the most striking examples of secondary sexual differences. In the first place monogamy, at least when the sexes are numerically equal, is the mating system least likely to develop sexual selection. In the second place, and more important, observations on bird behaviour have shown that much of the display of birds occurs after pairing, when competition must have ceased. Such sexual differences are concerned, either with inducing the female to copulate, or with maintaining the association of the sexes as long as it is necessary for the rearing of the young.

Huxley therefore introduced the term *epigamic* to apply to characters which increased the fertility of a given mating and therefore had a selective value for the species as a whole. Epigamic selection includes the major part of what Darwin meant by sexual selection. It also includes selection for characters to which Darwin did not refer, such as the structure of copulatory organs, sex differences in frequency of crossing over, and the XY mechanism. It is only a special case of natural selection as generally understood. What remains of Darwinian sexual selection has been called *intra-sexual selection*, which denotes that it involves competition between members of one sex for mates. It can only indirectly affect the survival of the species and then is often deleterious (*e.g.* the cumbersome antlers of the stag). There is not invariably, however, a clear distinction between epigamic and intra-sexual selection. In a promiscuous species like *Drosophila* pairing and copulation are synchronous. Courtship behaviour determines the number of mates and therefore enters into intra-sexual selection.

Intra-sexual selection has been demonstrated in *Drosophila* a number of times in the shape of sexual isolation between species, geographic races and mutants. Table 1 gives some typical examples.

TABLE 1

Examples of sexual isolation in Drosophila spp. *arranged in descending order of magnitude*

Isolation between	Authority
D. pseudoobscura and *D. miranda*	Dobzhansky and Koller, 1938
D. pseudoobscura strains *A* and *B*	" " " "
Certain mutants of *D. pseudoobscura A*	Tan, 1946
Yellow mutant and wild type of *D. subobscura*	Rendel, 1944
Geographic races of *D. sturtevanti*	Dobzhansky, 1944
Geographic races of *D. melanogaster*	Mather unpublished
Selection lines of *D. melanogaster*	Mather and Harrison, 1948

The standard technique is to enclose one kind of male with two kinds of female, one of them the same as the male, and then to observe the relative frequency of insemination of the two kinds of female.

Now it is generally assumed that intra-sexual selection almost invariably involves competition between males, the females exercising choice, and not the reverse. The presence of secondary sex differences does not allow one to decide the issue, since there is no *a priori* reason for assuming one sex to be primitive and the other derivative. It would be conceivable that both sexes had deviated equally from an unspecialised ancestral type. The assumption that it is the males which are mainly subject to the intra-sexual selection is in fact based mainly on the *behaviour* of animals. Darwin took it as a matter of general observation that males were eager to pair with any female, whereas the female, though passive, exerted choice. He was at a loss, however, to explain this sex difference, though it is obviously of great importance for an understanding of intra-sexual selection.

Drosophila seems to be no exception to the rule. In the paper cited above, Rendel observed courtship in *D. subobscura*. It is the male which makes the advances to any female and often even to other males, and it is the female which accepts or rejects the advances. This observation was supported by the peculiar effect of the mutant *yellow* as shown in reciprocal matings :—

yellow ♀♀ × + ♂♂ 100 per cent. inseminated
+ ♀♀ × *yellow* ♂♂ 2 per cent. inseminated

The males courted equally vigorously in both matings. Thus wild type females found *yellow* males objectionable, but wild type males failed to discriminate between *yellow* and wild type females. Females differed genetically in their discrimination against *yellow* males and it was possible by selection to obtain wild type strains which gave

fully effective matings with *yellow* males. A similar sex difference in effect of *yellow* on mating behaviour has been observed in *D. pseudoobscura* by Tan (1946). Other mutants affecting mating behaviour in this species were *aristapedia*, which did not affect males but reduced the mating capacity of the females (possibly by interfering with their ability to detect courting males); and the *Bare Curly* combination, which again did not affect males but enhanced the mating capacity of females. (Perhaps *Curly* females were unable to interpose their wings between their abdomina and approaching males.) The actions of all these mutants stress that it is the female which exercises the choice.

Nevertheless there is some evidence of discrimination by males. Stalker (1942) using the two subspecies *virilis* and *americana* of *D. virilis* found that the males of one subspecies ignored females of the other and were actually courted by them. It is to be expected of course, that if males are enclosed with sufficiently unrelated females they will show discrimination. Stalker's case is remarkable, however, in that the males showed more discrimination than the females of the other subspecies. Dobzhansky and Koller (1938) carried out a test for discrimination by males. Males of *D. pseudoobscura* which had been kept with their own females for five days and males which had been kept isolated from females for the same time were introduced to mixtures of *D. pseudoobscura* and *D. miranda* females. In both cases all the *D. pseudoobscura* females were inseminated, but the previously isolated males inseminated the higher proportion of *D. miranda* females. This was interpreted to mean that the isolated males had greater sexual appetites and were therefore less discriminating. A second possibility, which again involves the assumed capacity of males to discriminate, would be that males accustomed to mate with *D. pseudoobscura* females would show a greater preference for the same type when given choice than males with no previous experience. A third possible explanation not involving male discrimination was admitted. This was that the males which had been isolated might have inseminated the females of both species more frequently, but, as multiple inseminations could not be detected, the additional matings would only be noticed in the *D. miranda* females most of which remained unmated.

Dobzhansky has been most careful throughout his papers not to commit himself on the matter of which sex is exercising the discrimination. Tan (1946), on the other hand, interprets his data by assuming without evidence that it is the males which discriminate. In the now standard experiments on discrimination one kind of male is enclosed with two kinds of female. (As the observations are made on the ventral tubes of the females this arrangement is unavoidable until a new technique is perfected.) It is easy to fall into the error of assuming that it is the male which " chooses " between two kinds of female : the results could equally well be interpreted on the assumption that the two kinds of females discriminate with specific strengths against the same males.

Thus, so far as the evidence from observations on behaviour goes, it would appear that though discrimination is sometimes found in males, it is almost confined to the females. In other words intra-sexual selection is predominantly intra-masculine. Widespread though this sex difference in discrimination is generally understood to be, it does not appear to be a primary feature of sexual reproduction : it must be supposed to have evolved as a secondary sexual difference. In so far as the so-called secondary sex characters have arisen through the action of sexual discrimination, which is itself only secondary, they must be regarded as strictly tertiary.

The mating system of a species was considered by Darwin to be an important element in determining sexual selection. The only mating system in which intra-sexual selection is ineffective is strict monogamy with numerical equality of the sexes. (Monogamy implies that the mating group in any one breeding season is a single pair. It is immaterial from the point of view of sexual selection whether or not the same pair is mated for life.) All other systems, polygyny, polyandry, promiscuity, and numerical inequality of the sexes alone, will allow intra-sexual selection.

Darwin supposed that intra-masculine selection predominated because it was produced by the commonest mating systems. These were, monogamy combined with an excess of males, and polygyny (he used the ambiguous term " polygamy "). But why, then, should not the two corresponding systems inducive of intra-feminine selection, namely monogamy with excess of females and polyandry, be equally common ? It must be that only those mating systems compatible with intra-masculine selection have arisen. Consequently one must assume that intra-masculine selection determines the mating system and not, as Darwin supposed, that the mating system determines intra-masculine selection.

It is thus desirable to search for a fundamental cause of intra-masculine selection, independent of mating system and probably inherent in the mechanics of sexual reproduction. This same cause should show us why it is a general law that the male is eager for any female, without discrimination, whereas the female chooses the male. The experiments to be described concern a species, *Drosophila melanogaster*, in which the sexes are numerically equal and mating is promiscuous. Though discrimination between races has been demonstrated (Mather and Harrison unpub.) it is less marked than in some of the species referred to above. This species would not, therefore, appear at first sight to be particularly favourable material for demonstrating intra-sexual selection. Even in this species, however, as the results will show, sexual selection, if not confined to the males, preponderates in them. The results also indicate the cause of this sex difference in intensity of selection.

METHODS

The experiments use in a novel way the relation between selection and fertility. The fertility of an individual is here taken to mean its actual contribution to the next generation, rather than, as is sometimes meant, its potential contribution. The basic assumption made is that for a given genetic variation the variation in fertility increases with selection. Variance in fertility is in fact a measure of the intensity of selection.

The paradoxical relation between selection and fertility may be considered further. On the one hand selection, by eliminating certain genotypes, reduces the genetic variation of a population (including genetic variation in fertility). On the other hand selection causes an immediate increase in the phenotypic variation in fertility. As selection proceeds and genetic uniformity increases, the phenotypic variation in fertility will of course gradually decrease again. When selection is relaxed the immediate result will be a rapid further decrease in phenotypic variation in fertility, whereas genetic variation will only increase gradually as a result of mutation and recombination.

To return to the main argument, a sex difference in variance of fertility is therefore a measure of the sex difference in intensity of selection, which indicates that intra-sexual selection is greater in one sex than the other.

The fertility of individual flies of both sexes was measured by means of dominant marker genes. Several flies of each sex were mated together in one bottle, each fly carrying a different dominant marker gene. In this way, assuming the complete viability of all the marker genes, half the progeny of each fly could be identified. Any effects of differential viability of the marker genes can be isolated and removed in the experiments involving reciprocal matings. The identifiable progeny were used to estimate the variances in fertility of their parents.

Moreover one quarter of the progeny of each fly will carry two marker genes, one from each parent. In this way it is possible to determine which matings have taken place, and in how many inseminations each fly has participated. The number of inseminations identified should, however, be regarded as a minimum, for two reasons : the possibility that some matings might be ineffective, and the inability to distinguish single and multiple inseminations involving the same pair of flies.

First, it may not be possible to identify all the males by which the female has been inseminated. According to a number of authors (Nachtsheim, 1927 ; Dubinin, 1928 ; Kaufmann and Demerec, 1942), when a female is inseminated twice, the second batch of sperm supersedes the first which only reappears in the progeny when the second batch has been consumed. Thus it might be possible when two matings occurred in quick succession for no progeny from the

first mating to appear. Lobashov (1939) on the other hand claimed to get complete mixture of sperm. Some of the data enable one to assess the likelihood that replacement of one kind of sperm by another might interfere with the results. In series 5 (see table 3) the mated flies were transferred each day for 4 days to new bottles. In this way it was possible to observe whether a female producing progeny derived from the sperm of two males utilised the two batches of sperm separately or together.

Kind of sperm . . .	1st	2nd			
Day when first used . .	1st	1st	2nd	3rd	4th
Mixing of sperm . .	11	4	6	1	...
No mixing . . .	3	...	1	1	1

Evidently if the second insemination occurs soon after the first there is complete mixing of sperm, but as the interval of time between inseminations increases the second insemination becomes more likely to supersede the first. A mating would escape detection only when a second occurred a short time after the first and there was no mixing of sperm. The evidence is that this is rare.

An examination of the papers referred to above supports this conclusion. Kaufmann and Demerec introduced the second male 3 days after the first and Nachtsheim allowed 8 days to elapse before allowing a second mating. If Lobashov (whose original paper is not available) used a shorter interval the contradictions between their results would be resolved.

The second source of error is that one cannot measure directly the number of inseminations, but only the number of mates. The number of mates need not of course equal the number of inseminations, though it can never be greater. If, however, mating is unassortative and there is no tendency to monogamy, the discrepancy between the number of mates and the number of inseminations will be slight, unless the number of mates per fly is large. The average number of mates per fly varied from 1 to 1·9, whereas the maximum possible number was in most cases 3, sometimes 5. The error introduced by regarding the number of mates per fly as the number of inseminations will not, therefore, be serious.

The marker genes used in the experiment were extracted from various laboratory stocks. They are listed and described briefly in table 2. Some (*Pm*, *Cy*, *CyL*^{4}, *Sb*, *Mé*) marked inversions. The rest (*B*, *Bl*, *H*, *Mc*) were unaccompanied by inversions. The extracted mutants were at first kept in mass cultures by mating marked females to wild type males from the same stock. These stocks would

contain a large amount of internal genetic variability. As it was desired to reduce to a minimum the genetic causes of variability in fertility, all these stocks were later crossed to *Or*+*inbred*, a stock which had been maintained by brother-sister mating for over 200 generations. They were then backcrossed to *Or*+ for several generations using females carrying the marker genes, during which time the genetic variability within stocks must have become very low and the stocks would differ from one another mainly by the marker genes and the

TABLE 2

Description of the dominant marker genes used in the experiments

Chromosome	Symbol	Name	Main effect
I	*Hw*	Hairy-wing	Extra long bristles on wing veins. Homozygote viable and more extreme.
	B	Bar	Reduction of size of eye which becomes a narrow kidney shape. Homozygote fully viable and more extreme.
II	*Pm*	Plum	Eye colour brown: slight darkening of body colour. Homozygote lethal.
	Cy	Curly	Wings curled upwards. Homozygote lethal.
	CyL^{4}	Curly-Lobe	*Cy* with, in addition, eye reduced in size and with a nick in the ventral edge.
	Bl	Bristle	All bristles shortened and thickened. Homozygote lethal.
III	*Sb*	Stubble	Same as *Bl*. *Sb*+*Bl* more extreme than either.
	Mé	Moire	Eyes paler than wild type with shimmering appearance of shot silk. Body colour paler. Homozygote lethal.
	H	Hairless	Hairs removed from various parts of the body, particularly the post verticals at back of head. Homozygote lethal.
	Mc	Microcephalous	Eyes reduced or absent. Homozygote viable.

parts of the chromosomes closely linked with them. As a result of this backcrossing the fertility fell sharply and the flies were in effect monogamous. The males may in fact have been incapable of more than one fertile copulation. This was useless for the purposes of the experiment, so fertility and mating frequency were restored but uniformity retained by crossing these lines to another inbred line, the *Samarkand*+*inbred*. The parents for the final series of experimental matings were then F_1s between the two inbred stocks.

Full identification of flies carrying two marker genes was not always possible because of interaction between them. In the earlier matings when five flies of each sex were used, the females carried *Hw*, *Pm*, *Sb*, *Mé* and *H* while the males carried *B*, *Cy*, *CyL*^{4}, *Bl* and *Mc*. The former group of genes was called *C* and the latter *D*. *Bl* and *Sb* were indistinguishable from one another unless they were in combination with another marker. If a fly which might be *Bl* or *Sb* also carried a marker from group *D* the first mutant must have been from group *C* (i.e. *Sb*). When *Bl* and *Sb* occurred together a distinct

phenotype developed more extreme than either. The fertility of *Bl* and *Sb* flies was calculated by assuming that the ratio of simple *Bl* to simple *Sb* was the same as the ratio of combined *Bl* to combined *Sb* and dividing the unclassifiable flies between *Bl* and *Sb* accordingly.

Another complication arose through the interaction of *Pm* and *Mé* with *Mc*. It is impossible completely to score *Mc* flies for eye colour, as some of them are eyeless. This would have the effect of reducing the apparent fertility of *Pm* and *Mé* genotypes, but only when the fertility of a mating between *Mc* and *Pm* or *Mé* was very low could the mating be overlooked as a result of this interaction.

In later experiments the number of flies of each sex was reduced to three, the markers being *Pm*, *H* and *Sb* (group *A*) and *CyL*^{4}, *Cy* and *Mc* (group *B*). To minimise error variation in any series of matings all the flies of one sex were kept in the same sexing tube

TABLE 3

The distinctive features of the various series of matings

Number of series	♀×♂	Number of matings	Number of flies of each sex per mating	Number of days laying	Age relationship	Pedigree of parents
1	C×D	5	5	3	O, M and N mixed at random	Mass cultures
2	C×D	9	5	3	3 bottles each of O, M and N	Mass cultures
3	B×A	9	3	4	All 9 combinations of O, M and N. Flies of same sex in a bottle all of same age	Mass cultures
4	A×B	8	3	3	2 bottles of O, 3 of M and N.	Crossed to *Or*+ for 3 generations
	B×A	9	3	3	3 bottles of O, M and N	
5	A×B	4	3	4*	All M	F_1 between *Skd*+ and backcross to *Or*+ after 6 generations
	B×A	4	3	4*		
6	A×B	8	3	3	All M	F_1 between *Skd*+ and backcross to *Or*+ after 15 generations
	B×A	8	3	3		

Skd+ = Inbred stock from Samarkand wild type.
Or + = Inbred stock from Oregon wild type.

A = *Pm*, *H* and *Sb*.
B = *CyL*, *Cy* and *Mc*.
C = *Hw*, *Pm*, *Sb*, *H* and *Mé*.
D = *B*, *Cy*, *CyL*, *Bl* and *Mc*.

O = Flies mated at 6 days old.
M = Flies mated at 3 days old.
N = Flies mated at 1 day old.

* Flies transferred to new bottle every day.

until the day before mating. They were then grouped into sets of three or five and given a day to recover from the effects of ether before both sexes were introduced into the bottles. Copulating pairs

were frequently observed within a few minutes of introducing the sexes. The flies were allowed to lay 3 or 4 days. In one instance (series 5 of table 3) the flies were transferred to fresh bottles every day for 4 days.

The age of the flies was variable. There were three age groups according to the ages at which they were allowed to mate : 1, 3 and 6 days old. The distinctive features of the six series of matings are shown in table 3. An example of the way in which each mating was analysed is shown in table 4. In all, 220 flies of each sex were used as parents in the experiments.

TABLE 4

Sample result of one mating. From series 6 B×A, *sixth bottle* (*CyL, Cy, Mc*) × (*Pm, H, Sb*)

♂/♀	*CyL*	*Cy*	*Mc*	+	Total	No. of mates
Sb . .	13	0	0	16	**29**	1
Pm . .	10	12	15	68	**105**	3
H . .	7	29	0	41	**77**	2
+ . .	60	38	40	110	248	...
Total . .	**90**	**79**	**55**	235	459	...
No. of mates	3	2	1	...	...	...

Out of 459 flies emerging in one bottle, all but 110 carry markers, and 86 carry two markers. The mean squares for fertility of the six flies are based on the totals in heavy type.

RESULTS

As explained above, the intensity of selection is measured as the variability of fertility which is expressed as the mean square. The

TABLE 5

Overall mean squares for the two sexes compared for each series of matings

Series	Mating	Males		Females		Variance ratio	Probability
		Mean square	d.f.	Mean square	d.f.		
1	C×D	1377·8	19	474·9	19	2·90	**
2	C×D	734·6	44	183·9	44	3·99	***
3	B×A	2433·0	26	858·5	26	2·83	**
4	A×B	463·7	23	215·0	23	2·16	*
	B×A	1367·7	26	454·5	26	3·01	**
5	A×B	1604·4	11	984·6	11	1·63	...
	B×A	1700·4	11	208·9	11	8·14	***
6	A×B	2798·4	23	992·7	23	2·82	**
	B×A	1098·0	23	276·7	23	3·97	***

Probability * = ·05 or less. ** = ·01 or less. *** = ·001 or less.

mean squares showing the gross variability between flies of the same sex in each series are shown in table 5. In every case, including

those where reciprocal matings were used, the mean square for males is greater than that for females. The variance ratios between the two sexes vary from 1·6 to 8·1 and in most cases are highly significant. In series 1-3 it might be argued that the stocks used for males were more variable in their effect on fertility than those chosen for females. In all other series, where reciprocal matings were made, that possibility has been eliminated.

A second possibility is that the fertility of males is more sensitive to environmental effects such as age. This can be tested in series 1 and 3. In series 1, the ages of flies in a bottle varied at random. In males the mean square due to age is 20·5 whilst in females it is 424·6. There is a probability of 5 per cent. that this difference is random. Thus if there is any sex difference here, it is the females which are the more sensitive to age. In series 3 the variation between bottles is analysable into that due to age of males, age of females and error variation. Age is without effect in both sexes.

A third possibility is that males are inherently more sensitive to changes in genotype. The best test of this is the variation in fertility

TABLE 6

Analysis of variance showing mean squares for effect of marker and error

Series	Mating	Sex	Marker		Error		Variance ratios					
			M.S.	d.f.	M.S.	d.f.	Marker / Error	P.	Marker ♂/♀	P.	Error ♂/♀	P.
1	C×D	♂	2537·6	4	1180·9	12	2·15	...	3·02	...	3·48	*
		♀	838·9		339·5		2·47	...				
2	C×D	♂	2919·5	4	424·9	24	6·87	***	5·58	*	3·76	***
		♀	523·6		113·1		4·63	**				
3	B×A	♂	754·4	2	2317·8	12	0·33	...	0·41	...	4·15	**
		♀	1841·8		558·6		3·30	*				
4	A×B	♂	1545·1	2	501·9	10	3·08	...	0·77	...	7·05	***
		♀	2011·7		71·2		28·25	***				
	B×A	♂	4080·2	2	1564·7	12	2·61	...	4·88	...	5·31	**
		♀	836·2		294·6		2·84	...				
5	A×B	♂	2453·2	2	1976·9	6	1·24	...	0·55	...	9·28	**
		♀	4459·0		213·0		20·93	**				
	B×A	♂	3273·5	2	1930·5	6	1·70	...	7·52	...	29·25	***
		♀	435·2		66·0		6·59	*				
6	A×B	♂	5254·1	2	3700·7	14	1·42	...	1·25	...	4·43	**
		♀	4200·8		836·0		5·02	*				
	B×A	♂	348·0	2	1676·2	14	0·21	...	0·66	...	4·94	**
		♀	528·6		339·6		1·56	...				
Weighted Mean		♂	2602·1	22	1568·8	112	1·66	*	1·68	...	4·88	***
		♀	1548·9	22	321·6	112	4·82	***				

Probability * = ·05 or less. ** = ·01 or less. *** = ·001 or less.

between marker genes as measured by the mean square. Table 6 shows the analysis of variance of all the data into the mean squares

due to markers and to error. The error mean square represents that variation between individuals which remains after the variation due to all analysable causes (*i.e.* marker, age, and overall variation between bottles) has been extracted.

The mean square for markers is not consistently higher for males than for females. The comparison can be made, where there are reciprocal matings, either between sexes with the same genotypes or between sexes in the same mating. The results are the same whichever way the comparison is made. The significance of the female marker mean squares is actually much the greater because the corresponding error mean squares by which they are judged are always the smaller. One can at least conclude that the males are not sufficiently sensitive to genotypic changes to account for the sex difference in variance of fertility.

It should be noted that the parents of the first three series were from mass cultures, whereas in later series they were either inbreds or first crosses between inbreds. If genes other than the markers were having an important influence on male fertility the error mean squares in the first three series should be higher than in the second three. This is not apparent.

The wide variation between series in their error mean squares is not surprising since there is variation in the markers used, the age of the parents, their pedigree, and the laying conditions. The overall picture is quite definite, however. In every series the error mean square is higher in the male than in the female, whether the comparison is made between flies with the same markers or from the same mating. Every variance ratio is greater than the level required for the 5 per cent. level of significance, in most series much greater.

The results can be summarised by pooling all the mean square data in table 6. The sums of squares in each category are summed and divided by the total number of degrees of freedom. The mean squares so obtained are at the foot of the table. Though they are produced by combining heterogeneous data they can be seen to agree with the general trend. If the pooled marker mean squares are compared with their errors the males are at the 5 per cent. level of significance and in the females the ratio is much more significant. The ratio between error mean squares for the two sexes is highly significant, the males having the higher value. The ratio between marker mean squares would indicate that, if anything, the males are more sensitive to differences between marker genes. This greater sensitivity, however, is quite insufficient to account for the overall sex difference in mean squares, and in any case is not statistically significant.

It has now been demonstrated that the sex difference in variance of fertility is not due to the greater sensitivity of males to classifiable changes, environmental or genetic. The clue to this sex difference must be sought in the mechanism of sexual reproduction itself.

Further detailed examination of the results showed that whereas only 4 per cent. of the females were unrepresented in the progeny, 21 per cent. of the males were unrepresented (bottles in which any parent had died during the laying period were discarded). Since the males as a whole must mate as frequently as the females, if many males fail to mate there must be a similar number who mate excessively. Statistically speaking, the variance in number of mates is higher in males than in females. Table 7 shows the distribution of mates per

TABLE 7

The distribution of number of mates per fly in the two sexes

Series	Mating	Sex	No. of mates per fly						Mean	Mean square
			0	1	2	3	4	5		
1	C×D	♂	6	5	5	3	1	...	1·4	1·51
		♀	1	10	9	...	...	...		0·36
2	C×D	♂	13	8	13	9	2	...	1·5	1·48
		♀	1	22	19	3	...	...		0·44
3	B×A	♂	6	4	11	6	...	...	1·6	1·17
		♀	1	11	12	3	...	...		0·55
4	A×B	♂	5	13	6	...	...	...	1·0	0·48
		♀	1	21	2	...	...	...		0·13
	B×A	♂	10	8	8	1	...	...	1·0	0·85
		♀	3	21	3	...	...	...		0·23
5	A×B	♂	...	7	4	1	...	...	1·5	0·45
		♀	...	6	6	...	...	...		0·27
	B×A	♂	1	4	4	3	...	...	1·75	0·93
		♀	...	4	7	1	...	...		0·39
6	A×B	♂	4	11	4	5	...	...	1·4	0·96
		♀	1	12	11	...	...	...		0·27
	B×A	♂	...	8	11	5	...	...	1·9	0·55
		♀	...	6	15	3	...	...		0·38

fly in the six series, together with the mean squares for number of mates. This discloses an obvious clue to the high variance in fertility of males. One has only to assume that there is a relation between the number of mates of an individual and its fertility. These results can also be expressed in terms of sexual selection. There is greater competition for mates between males than between females, which confirms that the sex difference in selection intensity is due to intra-masculine selection. The pressure of this competition results in a high variability in number of matings. This intra-masculine competition is in fact that which was recognised in the introduction as the true secondary difference through which all so-called secondary sex differences are derived.

There is yet a further cause of the sex difference in fertility variance, which can be recognised if the mean fertility according to number of mates is estimated. These figures are given in table 8. It will now be observed that the fertility of the males increases steadily with the number of mates. There is a much weaker correlation

between fertility of females and number of mates, especially if we exclude those which have not mated at all. To facilitate comparison the fertilities have been expressed as a proportion of that obtaining with a single mate. The data can then be combined without a bias being introduced owing to the differential fertility of the separate series. In combining the ratios they are weighted according to the factor $\frac{n_1 n_2}{n_1+n_2}$, where n_1 and n_2 are the number of observations on which the numerator and denominator of the ratio are based. This

TABLE 8

The variation in fertility with the number of mates other than none

Series	Mating	Males (number of mates)				Females (number of mates)		
		1	2	3	4	1	2	3
1	C×D	28·2	38·4	85·3	84·0	26·7	39·2	...
		1·0	1·36	3·02	2·98	1·0	1·47	...
2	C×D	16·3	16·8	63·7	55·0	16·8	24·2	41·0
		1·0	1·03	3·91	3·37	1·0	1·44	2·44
3	B×A	19·8	69·7	97·2	...	41·3	62·1	77·0
		1·0	3·52	4·91	...	1·0	1·50	1·86
4	A×B	32·7	46·0	...	...	27·8	38·5	...
		1·0	1·41	...	...	1·0	1·38	...
	B×A	35·4	70·0	81·0	...	40·0	45·7	...
		1·0	1·98	2·29	...	1·0	1·14	...
5	A×B	48·0	81·0	168·0	...	58·5	71·3	...
		1·0	1·69	3·50	...	1·0	1·22	...
	B×A	47·8	70·5	124·0	...	69·0	64·7	75·0
		1·0	1·47	2·59	...	1·0	0·94	1·09
6	A×B	49·5	93·3	130·8	...	79·5	66·3	...
		1·0	1·88	2·64	...	1·0	0·83	...
	B×A	35·6	72·0	111·2	...	69·8	61·8	78·7
		1·0	2·02	3·12	...	1·0	0·89	1·13
Combining all data . .		1·0	1·79	3·36	3·24	1·0	1·24	1·79
Combining series 1-4 .		1·0	1·76	3·83	3·24	1·0	1·42	2·17
Combining series 5 and 6		45·2	77·0	125·0	...	71·4	65·1	77·8
		1·0	1·70	2·77	...	1·0	0·91	1·09

The upper figures for each mating are the mean fertilities. The lower figures are these means expressed as ratios to the fertility with a single mate. All the data are combined together by means of their ratios. Series 5 and 6 are also combined together by means of their mean fertilities which are then converted to ratios.

is the standard method of weighting ratios. In this way the mean relative fertilities, at the foot of the table, are obtained. It appeared, however, that series 5 and 6 differed somewhat from the rest. Series 1-4 were therefore combined in the manner described above giving results also shown at the foot of the table. They are also shown graphically in fig. 1 (*a*). There is an obvious sex difference, the females not responding so markedly to increases above one in number of mates.

The difference is even more noticeable, however, in series 5 and 6. These are more comparable with one another than with the other series since they both involved the use of parents which were F_1's between inbred lines. The fertilities of the two series are also very similar, so that the fertilities themselves have been combined subject only to weighting according to the number of observations on which each is based. The ratios obtained from these combined fertilities are shown in table 8 and in fig. 1 (*b*). The males show direct proportionality between number of mates and fertility (as before). The females, provided they have been mated with at least once, show absolutely no effect of number of mates.

That the sex difference is more obvious in these series than in the rest, may be due to the reduced vigour of the latter. If the stock

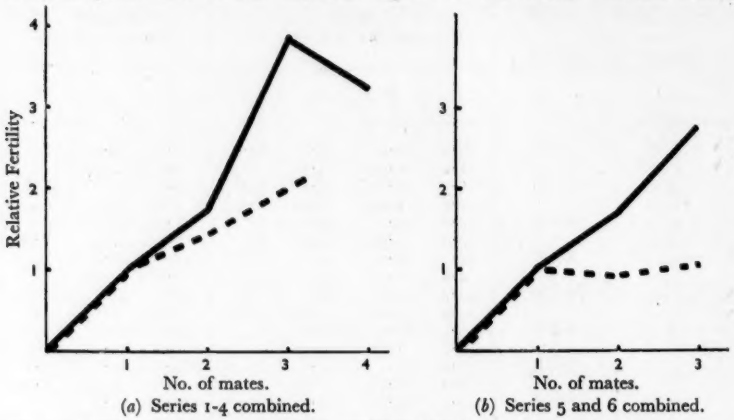

FIG. 1.—The relation between number of mates and fertility relative to that with one mate. Solid line, males : Broken line, females.

is weak, mating is less likely to occur immediately on introducing the sexes. There would then be a correlation between number of matings and earliness of the first mating which would itself be related to the fertility of the females. Again in a weaker stock, the sperm transferred per insemination may be insufficient to ensure full fertility over the 3 or 4 days of the experiment. This also would produce a correlation in females between number of mates and fertility.

It can now be seen that the sex difference in variance of fertility, which is itself a sign of intra-masculine selection, is due to the effect of number of mates per fly on fertility. This takes effect in two ways :—

(*a*) The higher variance, in males, of the number of mates per fly. This is a *sign* of intra-masculine selection.

(*b*) The stronger correlation, in males, between number of mates and fertility. This is the *cause* of intra-masculine selection.

To test whether these two factors offer a complete explanation of the sex difference, the sum of squares due to the effect of number of

mates can be subtracted from the total sum of squares in each series. If there are no other causes of the sex difference in variance, the remainder mean squares of the two sexes will be the same. This analysis is shown in table 9. There is a slight tendency for the male

TABLE 9

Variance of fertility analysed into that due to number of mates and the remainder

Series	Mating	Sex	Number of mates		Remainder		Variance ratios					
			M.S.	d.f.	M.S.	d.f.	Mates / Remainder	P.	Mates ♂/♀	P.	Remainder ♂/♀	P.
1	C×D	♂	4401·0	4	571·6	15	7·70	***	5·02	...	1·34	...
		♀	877·2	2	427·6	17	2·05	...				
2	C×D	♂	6091·1	4	199·0	40	30·61	***	8·22	*	1·39	...
		♀	740·9	3	143·1	41	5·18	**				
3	B×A	♂	12019·4	3	1182·6	23	10·16	***	5·12	...	1·78	...
		♀	2346·6	3	664·4	23	3·53	*				
4	A×B	♂	3057·6	2	216·6	21	14·12	***	6·12	...	1·15	...
		♀	499·9	2	187·9	21	2·66	...				
	B×A	♂	8050·3	3	496·1	23	16·23	***	3·57	...	1·63	...
		♀	2255·5	2	304·4	24	7·41	**				
5	A×B	♂	6732·0	2	464·9	9	14·48	***	13·62	...	0·45	...
		♀	494·1	1	1033·7	10	0·48	...				
	B×A	♂	5209·1	3	384·7	8	13·54	***	89·35	**	1·59	...
		♀	58·3	2	242·4	9	0·24	...				
6	A×B	♂	14787·3	3	1000·1	20	14·79	***	4·82	...	1·26	...
		♀	3067·7	2	795·1	21	3·86	*				
	B×A	♂	8946·2	2	350·6	21	25·52	***	21·45	*	1·33	...
		♀	417·0	2	263·3	21	1·58	...				
Combined data		♂	7678·4	26	524·0	180	14·65	***	6·05	***	1·32	*
		♀	1268·8	19	397·9	187	3·19	***				

Probability * ·05 or less. ** ·01 or less. *** ·001 or less.

remainder mean squares to be the higher, but this is never significant. Variance in number of mates is, therefore, the only important cause of the sex difference in variance of fertility.

DISCUSSION

It has been demonstrated that in *Drosophila melanogaster* sexual selection is much more effective in males than in females. This phenomenon is likely to occur also in other species of *Drosophila*. It might be expected to produce a situation in which males were less discriminating in their mating behaviour than females. This situation would mean that sexual isolation as demonstrated in *Drosophila* species by Dobzhansky and his co-workers, would be determined by discrimination on the part of the females rather than on that of the males.

The standard practice, however, of enclosing males of one kind

with females of two kinds, gives the latter no choice. It will disclose differences between strains of female only in the resistance they offer to a given strain of males. This would be compounded of their ability to discern whether males were of their own strain, their general mating propensity, and their ability to repel males with which they do not wish to mate.

An apparent outbreeding tendency could then be explained by a combination of weak discrimination with a strong mating propensity in the females of strain B when enclosed with females and males of strain A. It would seem desirable to carry out mating in which females were given a choice of males. Though there would be great technical difficulties, it would be informative to obtain comparative data from both types of mating.

On the assumption that all the discrimination is exercised by the females, further information can be obtained from the data on sexual isolation. The complementary matings [(A+B) ♀♀ ×A ♂♂ and (A+B) ♀♀×B ♂♂] then bear a special relation to one another. The degree of sexual isolation is measured by the isolation index which varies from +1 to −1. A positive index denotes a preponderance of intra-strain matings, a negative index a preponderance of inter-strain matings. The additional information is obtained from the sum of the isolation indices of complementary matings and from their difference. The sum denotes the degree of sexual isolation. The difference denotes the degree of non-specific sexual selection favouring females of one strain. Theoretically the sum could be negative though this would imply discrimination in favour of cross-breeding as distinct from the non-specific effect, which is unlikely. A study of the numerous data on the subject shows no evidence of this.

Beyond demonstrating the strength of intra-masculine selection in *Drosophila melanogaster*, the experiments described in the first part of this paper have also shown its cause, namely, the greater dependence of males for their fertility on frequency of insemination. Though this will clearly apply to all animals in which the female can store sperm, it can be shown that it is in fact an almost universal attribute of sexual reproduction.

In most animals the fertility of the female is limited by egg production which causes a severe strain on their nutrition. In mammals the corresponding limiting factors are uterine nutrition and milk production, which together may be termed the capacity for rearing young. In the male, however, fertility is seldom likely to be limited by sperm production but rather by the number of inseminations or the number of females available to him. In promiscuous species the share of males in the progeny of any female will be proportional to the number of inseminations for which each is responsible. In general, then, the fertility of an individual female will be much more limited than the fertility of a male.

The primary cause of intra-masculine selection would thus seem

to be that females produce much fewer gametes than males. Consequently there is competition between male *gametes* for the fertilisation of the female *gametes*. And this competition is vastly more intense than that hitherto considered between zygotes.

The primary feature of sexual reproduction is to be sure the fusion of gametes irrespective of their relative size, but the specialisation into large immobile gametes and small mobile gametes produced in great excess (the primary sex difference), was a very early evolutionary step. One would therefore expect to find in all but a few very primitive organisms, and those in which monogamy combined with a sex ratio of unity eliminated all intra-sexual selection, that males would show greater intra-sexual selection than females. This would explain why in unisexual organisms there is nearly always a combination of an undiscriminating eagerness in the males and a discriminating passivity in the females. Even in derived monogamous species (*e.g.* man) this sex difference might be expected to persist as a relic.

With intra-masculine selection males will be expected to show polygamous tendencies, whereas in females there would be selection in favour of obtaining only one mate after which they would become relatively indifferent. In *Drosophila* it has been shown that there is a high variance in number of mates in males, implying a polygamous tendency. Regarding the other sex Rendel (1944) observed that, immediately after one mating, females of *Drosophila subobscura* actually repelled courting males. It would appear then that polygyny has arisen as a result of intra-masculine selection.

On the other hand, one would expect to find polyandry only when special circumstances reduced the effect of normal selective forces. If there were an excess of males polyandry would not normally improve the fertility of the species since the number of females would be the limiting factor. Polyandry could, however, sometimes have a selective value. Thus in fishes, where fertilisation is external and very inefficient the sperm of several males may be necessary to fertilise all the eggs of one female.

Intra-masculine selection will often have the effect that a character which increases the chances of its possessors mating will be of epigamic value in females but only of value in intra-sexual selection in males. This situation can be seen in moths where the female has a scent to attract males. A female with a stronger scent than the rest would be found earlier and would lay its eggs sooner, but unless there were a severe food shortage there would be no adverse effect on the progenies of other less attractive females. On the other hand, a male with a stronger sense of perception would fertilise more females than his competitors and would reduce their chances of rearing progenies.

If the differentiation into male and female gametes is the basis of intra-masculine selection there should be signs of this selection in plants as well as in animals. Since plants are usually hermaphrodite and also sedentary, such selection would only be expected to show

in the pollen. The general tendency for the production of microspores far in excess of the minimum required to produce effective fertilisation is explicable in this way. In dioecious plants or moneocious plants where the sexes are separated the results of intra-masculine selection might be more obvious. A possible example here is the insect-pollinated sallow (*Salix caprea*) in which the male catkins are brightly coloured whereas the female catkins are inconspicuous (though both produce nectar).

The widespread distribution of intra-masculine selection would appear to be linked to another sex difference which has not yet been explained, namely, that when sex determination is by the XY mechanism, the male is usually the heterogametic sex. In animals the XY mechanism has become stabilised in large groups of common origin and has therefore probably arisen only a few times. The female is heterogametic in Lepidoptera, birds and a few fishes. The male is heterogametic in all other insects and vertebrates. In plants where dioecism has arisen independently on many occasions the position is even more indicative of a selective advantage in heterogametic males. Of the several species in which the X and Y have been distinguished, only one, *Fragaria elatior* has a heterogametic female. On the other hand *Rumex*, *Humulus*, *Mercurialis* and *Melandrium* are only the best known examples with heterogametic males.

In the early stages of differentiation between X and Y, the Y could act as a store of genes which were of intra-masculine selective value but deleterious in females. As differentiation proceeded by disorganisation of the Y and before dosage compensation became complete the heterogametic sex would be at a disadvantage owing to the functioning of genes in the hemizygous condition. A still greater handicap for the heterogametic sex is that when the Y is greatly disorganised and XY individuals are hemizygous for many genes the genetic balance normally obtained through the diploid condition is lost as far as the sex chromosomes are concerned. It has been shown that the loss of males can be compensated for by greater reproductive activity of the remaining males whereas the loss of females can not be made up so easily. Species with heterogametic males would therefore have an advantage over those with heterogametic females. The primary sex difference can therefore account for not only intra-masculine selection but also the sex distribution of the XY mechanism.

SUMMARY

1. The evidence on intra-sexual selection has so far indicated that it acts mainly in males, but it has given no adequate explanation why this should be so.

2. Experiments with *Drosophila melanogaster* using multiple matings in which each fly carries a different dominant marker, show that

the contribution of males to the next generation is more variable than that of females.

3. This sex difference is independent of environmental and heritable effects. Males must therefore be inherently subject to stronger selection than females, which must be due to a more intense intra-sexual action.

4. The intensity of intra-masculine selection is due to the greater dependence of the fertility of males on frequency of insemination. This seems to be inherent in primary sexual differentiation in both animals and plants.

5. Undiscriminating eagerness in males and discriminating passivity in females must have been early effects of intra-masculine selection and are naturally widespread. Previous experiments on sexual isolation in *Drosophila* can be interpreted on the assumption that females (not males) exercise discrimination.

6. Intra-masculine selection and related effects may have influenced the evolution of animals and plants in various ways.

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REFERENCES

DARWIN, C. 1871.
Descent of Man: Part II Sexual Selection.

DOBZHANSKY, TH. 1944.
Experiments on sexual isolation in *Drosophila*. III. Geographic strains of *D. sturtevanti*.
P.N.A.S. 30, 335.

DOBZHANSKY, TH., AND KOLLER, P. C. 1938.
An experimental study of sexual isolation in *Drosophila*.
Biol. Zent. 58, 589.

DUBININ, N. P. 1928.
Zh. Exp. Biol. 4, 131.

HUXLEY, J. S. 1938.
Darwin's theory of sexual selection.
Am. Nat. 72, 416.

KAUFMANN, B. P., AND DEMEREC, M. 1942.
Utilisation of sperm by the female of *Drosophila melanogaster*.
Am. Nat. 76, 445.

LOBASHOV, M. E. 1939.
C.R. Acad. Sci. U.R.S.S. 23, 827.

NACHTSHEIM, H. 1927.
Der Lebensdauer genotypisch verschiedener Spermien bie *Drosophila*.
Verh. V int. Kong. Vererb. Z.I.A.V. Suppl. II, 1143 (1928).

RENDEL, J. M. 1944.
Genetics and cytology of *Drosophila subobscura*. II. Normal and selective matings in *D. subobscura*.
J. Genet. *46*, 287.

STALKER, H. D. 1942.
Sexual isolation studies in the species complex *Drosophila virilis*.
Genetics *27*, 238.

TAN, C. C. 1946.
Genetics of sexual isolation between *Drosophila pseudoobscura* and *D. persimilis*.
Genetics *31*, 558.

THE ESTIMATION OF GENE FREQUENCIES FROM FAMILY RECORDS

II. FACTORS WITH DOMINANCE

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1. INTRODUCTION

In the previous paper (Finney, 1948), the theory of scoring records of related individuals, for the purpose of obtaining an estimate of gene frequency in the population from which the records were derived, was discussed. As Fisher (1940) has shown, application of the principle of maximum likelihood leads to a system of efficient scores, and so to an estimate of gene frequency which not only is unbiased but also has a minimal sampling variance. Formulæ for these scores have been derived and tabulated in the earlier paper for three types of record, two parents and their s children, one parent and one child, and two sibs with neither parent recorded, in respect of a genetic factor which shows no dominance. For sets of sibs with one or neither parent recorded, the complexity of the scores increases so rapidly as to make maximum likelihood scoring impracticable; a modification of a method due to Cotterman (1947), simple in use and of high efficiency, was therefore recommended for general use and tables of weights to be attached to the scores were given.

The purpose of the present paper is to set out a similar scoring system for use with a factor showing dominance. Fisher has already given results for maximum likelihood scores, and Cotterman has again pointed the way to a simplified system of high efficiency. Some recapitulation of these results is necessary for completeness here; the final system recommended is of higher efficiency than Cotterman's, but more easily applied to large families than Fisher's.

2. MAXIMUM LIKELIHOOD SCORING

A genetic factor involving two allelomorphic genes, T and t, of which T is dominant to t, gives rise to only two phenotypically distinguishable forms of individual. If the gene frequencies are denoted by μ, ν respectively, the frequency of recessive tt individuals in a population mating at random will be ν^2. It is convenient to develop formulæ for the estimation of ν^2, rather than of ν, since for the simplest case the procedure reduces to a count of recessives.

A sample of unrelated individuals consisting of a dominants and b recessives yields an estimate

$$\nu^2 = \frac{b}{a+b}, \quad . \quad . \quad . \quad . \quad (1)$$

with variance

$$V(\nu^2) = \frac{\nu^2(1-\nu^2)}{a+b} \quad . \quad . \quad . \quad . \quad (2)$$

For groups of related individuals, an estimate formed in this way will still be consistent and unbiased, but its variance will be greater than that given by equation (2). Furthermore, it will not in general be the most precise estimate obtainable from the data.

The theory of fully efficient scores, based on the principle of maximum likelihood, has been outlined by Fisher (1940) and Finney (1948). The probability that members of a family (as defined in the previous paper), having a specified pattern of relationship with one another, shall be of a particular observed set of phenotypes may be expressed as a function of ν, $P(\nu)$. A weighted maximum likelihood score for the family may then be calculated in terms of a provisional estimate of ν^2 as

$$x = W_L\nu^2 + \frac{\nu^2(1-\nu^2)}{P}\frac{dP}{d\nu^2}; \quad . \quad . \quad . \quad (3)$$

W_L, the weight of the score is given by

$$W_L = S\left\{\frac{1}{P}\left(\frac{dP}{d\nu^2}\right)^2\right\}, \quad . \quad . \quad . \quad . \quad (4)$$

the summation being over families of the same pattern with all possible sets of phenotypes. A revised estimate obtained from records of a number of families of any pattern is

$$\nu^2 = \frac{\Sigma x}{\Sigma W_L}, \quad . \quad . \quad . \quad . \quad (5)$$

with variance

$$V(\nu^2) = \frac{\nu^2(1-\nu^2)}{\Sigma W_L}, \quad . \quad . \quad . \quad . \quad (6)$$

Σ denoting summation over all records. The weight may be regarded as the number of unrelated individuals required to provide the same amount of information on ν^2 as is given by the family. Except for the occurrence of ν^2 instead of ν, these equations are the same as equations (3)-(6) of the previous paper, where they were derived for use with factors showing no dominance. Three examples follow.

(i) *Both parents recorded*

When both parents are recorded for a factor without dominance, children contribute no information (Cotterman, 1947 ; Finney, 1948). This is not so for a factor with dominance, since the phenotypes of

children may contribute information on whether a T parent is homozygous or heterozygous. For two parents with one child, the calculations leading to the maximum likelihood scores may be put in tabular form :—

Phenotypes		P	$\frac{dP}{d\nu^2}$	$\frac{1}{P}\frac{dP}{d\nu^2}$	$\frac{1}{P}\left(\frac{dP}{d\nu^2}\right)^2$
Parents	Child				
T, T	T	$\mu^2(1+2\nu)$	-3μ	$\frac{-3}{(1-\nu)(1+2\nu)}$	$\frac{9}{1+2\nu}$
T, T	*tt*	$\mu^2\nu^2$	$\mu(1-2\nu)$	$\frac{1-2\nu}{\nu^2(1-\nu)}$	$\frac{(1-2\nu)^2}{\nu^2}$
T, *tt*	T	$2\mu\nu^2$	$2-3\nu$	$\frac{2-3\nu}{2\nu^2(1-\nu)}$	$\frac{(2-3\nu)^2}{2\nu^2(1-\nu)}$
T, *tt*	*tt*	$2\mu\nu^2$	$\nu(3-4\nu)$	$\frac{3-4\nu}{2\nu^2(1-\nu)}$	$\frac{(3-4\nu)^2}{2\nu(1-\nu)}$
tt, *tt*	*tt*	ν^4	$2\nu^2$	$\frac{2}{\nu^2}$	4

Hence

$$S\left\{\frac{1}{P}\left(\frac{dP}{d\nu^2}\right)^2\right\} = \frac{6-\nu+\nu^2}{2\nu^2(1-\nu)(1+2\nu)},$$

so that, if $W_{a,L}(s)$ is the maximum likelihood weight for a family with a parents and s children recorded,

$$W_{2,L}(1) = \frac{(1+\nu)(6-\nu+\nu^2)}{2(1+2\nu)} \quad . \quad . \quad . \quad (7)$$

The weight is always greater than 2 (and rises to 3 when ν is very small) ; this is because the child provides evidence on the genotypes of the parents, so making the record of greater value than if the parents were scored as unrelated individuals and the child discarded.

From equation (3) and the working table above, the scores are derived as :—

$$\left.\begin{aligned}
&\text{Parent T, T ; child T} : x = -\frac{\nu^3(1-\nu^2)}{2(1+2\nu)}\\
&\text{Parents T, T ; child } tt : x = \frac{(1-\nu^2)(2+2\nu-\nu^3)}{2(1+2\nu)}\\
&\text{Parents T, } tt \text{ ; child T} : x = \frac{(1+\nu)(2+\nu-\nu^3+\nu^4)}{2(1+2\nu)}\\
&\text{Parents T, } tt \text{ ; child } tt : x = \frac{(1+\nu)(3+2\nu-2\nu^2-\nu^3+\nu^4)}{2(1+2\nu)}\\
&\text{Parents } tt, tt \text{ ; child } tt : x = \frac{(1+\nu)(4+4\nu-2\nu^2-\nu^3+\nu^4)}{2(1+2\nu)}
\end{aligned}\right\} \quad (8)$$

Table 1, analogous to Fisher's (1940) tables 1 and 3, gives numerical values for these scores and the weight, W, at different levels of ν^2.

This table may be seldom required, however, as the modified scoring system described in section 3 is also fully efficient for families with one child and has the advantage of being applicable for larger values of s.

TABLE 1

Maximum likelihood score and weight for two parents and one child

ν^2 (provisional)	Scores for phenotypes as shown (parents on first line, child on second)					W_L
	T, T / T	T, T / *tt*	T, *tt* / T	T, *tt* / *tt*	*tt*, *tt* / *tt*	
0·00	0·0000	1·0000	1·0000	1·5000	2·0000	3·0000
0·05	−0·0037	0·7995	0·9364	1·4114	2·0232	2·4631
0·10	−0·0087	0·7169	0·9251	1·3751	2·0332	2·3317
0·15	−0·0139	0·6506	0·9192	1·3442	2·0379	2·2525
0·20	−0·0189	0·5923	0·9159	1·3159	2·0395	2·1974
0·25	−0·0234	0·5391	0·9141	1·2891	2·0391	2·1562
0·30	−0·0274	0·4896	0·9134	1·2634	2·0373	2·1244
0·35	−0·0308	0·4430	0·9138	1·2388	2·0346	2·0990
0·40	−0·0335	0·3989	0·9152	1·2152	2·0314	2·0785
0·45	−0·0355	0·3570	0·9174	1·1924	2·0278	2·0618
0·50	−0·0366	0·3169	0·9205	1·1705	2·0240	2·0481
0·55	−0·0370	0·2786	0·9245	1·1495	2·0203	2·0369
0·60	−0·0365	0·2420	0·9293	1·1293	2·0166	2·0276
0·65	−0·0351	0·2069	0·9350	1·1100	2·0131	2·0202
0·70	−0·0329	0·1732	0·9416	1·0916	2·0099	2·0142
0·75	−0·0297	0·1410	0·9491	1·0741	2·0071	2·0094
0·80	−0·0257	0·1102	0·9574	1·0574	2·0046	2·0058
0·85	−0·0207	0·0807	0·9667	1·0417	2·0027	2·0031
0·90	−0·0147	0·0525	0·9769	1·0269	2·0012	2·0013
0·95	−0·0078	0·0256	0·9880	1·0130	2·0003	2·0003
1·00	0·0000	0·0000	1·0000	1·0000	2·0000	2·0000

This table is analogous to those given by Fisher (1940 ; tables 1 and 3) for one parent and one child and for a pair of sibs.

As usual, the complexity of the maximum likelihood scoring system is much greater for families of more than one child. The results for $s = 2$ have been obtained, but the scores have not been tabulated ; the weight, which is required in the examination of the efficiency of the linear scores in section 3, is

$$W_{2,L}(2) = \frac{(1+\nu)(60-70\nu+42\nu^2-19\nu^3+5\nu^4)}{2(2-\nu)(4+8\nu-3\nu^2)}. \quad . \quad (9)$$

When the number of children is large, their segregations for T give a considerable amount of evidence on the genotypes of the parents. The limiting result is

$$W_{2,L}(s) \to \frac{(1+\nu)(6-3\nu+\nu^2)}{1+3\nu} \text{ as } s \to \infty, \quad . \quad . \quad (10)$$

a quantity which ranges from 6 when $\nu = 0$ to 2 when $\nu = 1$.

(ii) One parent recorded

For a record consisting of one parent and one child, Fisher (1940) used a similar analysis to show that the scores are :—

$$\left.\begin{aligned}
&\text{Parent T, child T}: x = -\frac{\nu^3(1+\nu)(1-2\nu)}{4(1+\nu-\nu^2)}\\
&\left.\begin{aligned}\text{Parent T, child } tt\\ \text{Parent } tt\text{, child T}\end{aligned}\right\}: x = \frac{(1+\nu)(4-2\nu-2\nu^2-\nu^3+2\nu^4)}{4(1+\nu-\nu^2)}\\
&\text{Parent } tt\text{, child } tt: x = \frac{(1+\nu)(6-4\nu^2-\nu^3+2\nu^4)}{4(1+\nu-\nu^2)}
\end{aligned}\right\} \quad . \quad (11)$$

with a weight for the pair of individuals

$$W_{1,L}(1) = \frac{(1+\nu)(8-7\nu+2\nu^2)}{4(1+\nu-\nu^2)} \quad . \quad . \quad . \quad (12)$$

Fisher shows values of these functions in his table 1. Again no attempt has yet been made to investigate the more complex expressions required for several children with one recorded parent, except to obtain the weights for $s = 2$ and for large s :

$$W_{1,L}(2) = \frac{(1+\nu)(264-568\nu+488\nu^2-219\nu^3+65\nu^4-20\nu^5+4\nu^6)}{4(2-\nu)(3-\nu)(4+4\nu-7\nu^2+\nu^3)} \quad (13)$$

and

$$W_{1,L}(s) \to \frac{(1+\nu)(5-6\nu+3\nu^2-\nu^3)}{1+2\nu-2\nu^2} \text{ as } s \to \infty \quad . \quad . \quad (14)$$

The last expression shows that for a small value of ν a large number of sibs with one recorded parent are almost as informative as 5 unrelated individuals, but that for large ν the value falls to 2.

(iii) Neither parent recorded

For two sibs with neither parent recorded, Fisher obtained the scores * :—

$$\left.\begin{aligned}
&\text{Children T T}: x = W\nu^2 - \frac{\nu^2(1+\nu)(7-3\nu-2\nu^2)}{4+4\nu-3\nu^2-\nu^3}\\
&\text{Children T } tt: x = W\nu^2 + \frac{(1+\nu)(3-3\nu-2\nu^2)}{3+\nu}\\
&\text{Children } tt, tt: x = W\nu^2 + (1-\nu)(1+2\nu)
\end{aligned}\right\} \quad . \quad (15)$$

where the weight per sib-pair is

$$W_{0,L}(2) = \frac{(1+\nu)(21-5\nu-8\nu^2+4\nu^3)}{(3+\nu)(4+4\nu-3\nu^2-\nu^3)} \quad . \quad . \quad (16)$$

These functions have been tabulated by Fisher in his table 3 ; once

* In Fisher's formula for the score when both children are T, the factor $(7+3\nu-2\nu^2)$ is a misprint for $(7-3\nu-2\nu^2)$; the numerical values in his table 3 are calculated from the correct formula.

again corresponding expressions for larger sibships have not been derived, except for the limiting value

$$W_{0,L}(s) \to \frac{(1+\nu)(4-3\nu+2\nu^2-\nu^3)}{1+2\nu-\nu^2} \text{ as } \nu \to \infty, \quad . \quad (17)$$

the value of which ranges from 4 when $\nu = 0$ to 2 when $\nu = 1$.

3. SIMPLIFIED SCORING SYSTEMS

The property of full efficiency possessed by a maximum likelihood estimation process is of little value to the investigator if the formulæ required for its use are not known to him. An occasional record of a group of relatives not covered by the scoring methods of section 2 might be dealt with by developing the maximum likelihood equations from first principles as required, but the application of section 2 is so limited in respect of family size that some alternative procedure is much to be desired. Cotterman (1947) showed that a score which was a simple count of genes was of high efficiency for the estimation of ν^2. Just as in the previous paper (Finney, 1948), a sub-classification of types of record leads to a scoring system a little more efficient than Cotterman's which, with the aid of tables presented here, can be applied almost as easily as his very simple system. In this section, formulæ and tables will be given for sibships of s with two, one, or no parents recorded.

(i) Both parents recorded

Cotterman found that a score which is simply the total number of recessives amongst the $(s+2)$ individuals recorded carries the weight

$$W_{2,C}(s) = \frac{4(s+2)^2(1+\nu)}{(s^2+3s+8)+\nu(3s^2+17s+8)} \quad . \quad . \quad (18)$$

The weight for two unrelated individuals, scored by a simple count of recessives, is 2 ; when ν exceeds $(s+5)/(s+9)$, $W_{2,C}(s)$ is less than 2, and rejection of the children from the score will therefore improve the efficiency. By comparison of equation (18) for $s = 1$ with the maximum likelihood weight, equation (7), the efficiency of Cotterman's score is seen to be

$$\text{Efficiency} = \begin{cases} \dfrac{18(1+2\nu)}{(3+7\nu)(6-\nu+\nu^2)} & \text{for } \nu \leqslant 0{\cdot}6 \\ \dfrac{4(1+2\nu)}{(1+\nu)(6-\nu+\nu^2)} & \text{for } \nu > 0{\cdot}6 \end{cases} \quad . \quad (19)$$

This efficiency approaches unity when ν is very small or very large, and never falls below 0·95. A similar comparison of equation (18) for $s = 2$ with equation (9) gives the efficiency for families of two children ; in general this is rather less than for one child, but it

never falls below 0·92. The large family efficiency, in the limit as $s \to \infty$, is obtained from equations (10) and (18) as

$$\text{Efficiency} = \frac{4}{6-3\nu+\nu^2}; \quad . \quad . \quad . \quad (20)$$

the situation here is very different from that in the absence of dominance, as Cotterman's score is not fully efficient except for $\nu = 1$, and may involve a sacrifice of as much as one-third of the information when ν is small. These three efficiency functions are shown graphically in fig. 1.

The efficiency may be increased by scoring all parents as unrelated individuals and, separately, scoring children according to a system specially appropriate to their parental phenotypes. The derivation of scores for the children requires detailed analysis of possible sibships. The probability that a parent phenotypically T shall be heterozygous, in the absence of any information on children or other relatives, is $2\nu/(1+\nu)$. Hence the probabilities of the various phenotype combinations among the children of a T×T mating are :—

Parents	No. of children		P
	T	*tt*	
TT×T .	s	0	$1-\frac{4\nu^2}{(1+\nu)^2}$
Tt×Tt .	$s-n$	n	$\frac{4\nu^2}{(1+\nu)^2}\binom{s}{n}\frac{3^{s-n}}{4^s}$

If families of this type were scored with n, the total number of recessive children, the mean score would be

$$\begin{aligned} \mathrm{E}(n) &= \frac{4\nu^2}{(1+\nu)^2}\sum_{n=1}^{s} n\binom{s}{n}\frac{3^{s-n}}{4^s} \\ &= \frac{s\nu^2}{(1+\nu)^2} \quad . \quad . \quad . \quad . \quad . \quad . \quad (21) \end{aligned}$$

Summation of n^2 similarly leads to the variance

$$\mathrm{V}(n) = \frac{s\nu^2}{4(1+\nu)^4}\{s(1-\nu)(1+3\nu)+3(1+\nu)^2\} \quad . \quad (22)$$

Suppose now that ν_0^2 is a first approximation to the desired estimate, ν^2, and that

$$\nu^2 = \nu_0^2+\delta.$$

Define the score, y, as

$$y = -s\nu_0^3+n(1+\nu_0)^3 \quad . \quad . \quad . \quad . \quad (23)$$

Then

$$\mathrm{E}(y) = s(\nu_0^2+\delta) \quad . \quad . \quad . \quad . \quad . \quad (24)$$

to the first order in δ, and

$$\mathrm{V}(y) = \tfrac{1}{4}s\nu^2(1+\nu)^2\{s(1-\nu)(1+3\nu)+3(1+\nu)^2\}.$$

Consequently the information on ν^2 provided by the score is

$$i(\nu^2) = \frac{4s}{\nu^2(1+\nu)^2\{s(1-\nu)(1+3\nu)+3(1+\nu)^2\}} \quad . \quad (25)$$

For a T×*tt* mating with s children, similar analysis shows that the mean number of recessive children is

$$\mathrm{E}(n) = \frac{s\nu}{1+\nu}, \quad . \quad . \quad . \quad . \quad (26)$$

with variance

$$\mathrm{V}(n) = \frac{s\nu}{2(1+\nu)^2}\{s(1-\nu)+(1+\nu)\} \quad . \quad . \quad . \quad (27)$$

Taking ν_0^2 as before, a score

$$y = -s\nu_0^2(1+2\nu_0)+2n\nu_0(1+\nu_0)^2 \quad . \quad . \quad . \quad (28)$$

has expectation

$$\mathrm{E}(y) = s(\nu_0^2+\delta), \quad . \quad . \quad . \quad . \quad (29)$$

to the first order in δ, and variance

$$\mathrm{V}(y) = 2s\nu^3(1+\nu)^2\{s(1-\nu)+(1+\nu)\}.$$

The information on ν^2 given by this score is therefore

$$i(\nu^2) = \frac{s}{2\nu^3(1+\nu)^2\{s(1-\nu)+(1+\nu)\}} \quad . \quad . \quad (30)$$

A mating of *tt*×*tt* can produce only *tt* children, and these clearly provide no additional information on gene frequencies. Fisher (1940) has pointed out that a scoring of all families of two recorded parents with an average weight has the apparently irrational effect of increasing the weight assigned to the record of a *tt*×*tt* mating for each additional child, in spite of the fact that all children must be *tt*. The objection is not serious, but it is perhaps preferable to score these children as zero with zero weight. For the remaining families, the mean information per family is :

$$i(\nu^2) = \frac{1-\nu^2}{1+\nu^2} \times \text{Information for } (\mathrm{T}\times\mathrm{T}) + \frac{2\nu^2}{1+\nu^2} \times \text{Information for } (\mathrm{T}\times tt)$$

Hence the mean weight per individual scored in the sibships is

$$\left.\begin{aligned} &\text{Parents T, T, or T, } tt\text{:} \quad w = \frac{4(1-\nu)^2}{(1+\nu^2)\{s(1-\nu)(1+3\nu)+3(1+\nu)^2\}} \\ &\qquad\qquad + \frac{\nu(1-\nu)}{(1+\nu)(1+\nu^2)\{s(1-\nu)+(1+\nu)\}} \\ &\text{Parents } tt,\ tt\text{:} \quad w = 0 \end{aligned}\right\} \quad (31)$$

and the scores are

$$\left.\begin{aligned} &\text{Parents T, T:} \quad y = -s\nu^3+n(1+\nu)^3 \\ &\text{Parents T, } tt\text{:} \quad y = -s\nu^2(1+2\nu)+2n\nu(1+\nu)^2 \\ &\text{Parents } tt,\ tt\text{:} \quad y = 0 \end{aligned}\right\} \quad (32)$$

where s is the total number of children, of whom n are recessive. The various functions required for scoring with any trial value of ν^2 are given in table 2, and the weight per sib, w, is tabulated against ν^2 and s in table 3.

TABLE 2

Functions required in forming most efficient linear scores for children of two recorded parents

ν^2	ν^3	$(1+\nu)^3$	$\nu^2(1+2\nu)$	$2\nu(1+\nu)^2$
0·00	0·0000	1·0000	0·0000	0·0000
0·05	0·0112	1·8320	0·0724	0·6696
0·10	0·0316	2·2803	0·1632	1·0957
0·15	0·0581	2·6700	0·2662	1·4908
0·20	0·0894	3·0311	0·3789	1·8733
0·25	0·1250	3·3750	0·5000	2·2500
0·30	0·1643	3·7075	0·6286	2·6241
0·35	0·2071	4·0319	0·7641	2·9973
0·40	0·2530	4·3504	0·9060	3·3709
0·45	0·3019	4·6643	1·0537	3·7454
0·50	0·3536	4·9749	1·2071	4·1213
0·55	0·4079	5·2828	1·3658	4·4990
0·60	0·4648	5·5886	1·5295	4·8787
0·65	0·5240	5·8927	1·6981	5·2605
0·70	0·5857	6·1956	1·8713	5·6446
0·75	0·6495	6·4976	2·0490	6·0311
0·80	0·7155	6·7988	2·2311	6·4199
0·85	0·7837	7·0995	2·4173	6·8112
0·90	0·8538	7·3999	2·6076	7·2050
0·95	0·9259	7·7000	2·8019	7·6012
1·00	1·0000	8·0000	3·0000	8·0000

Scores are calculated from s, the total number of sibs, and n, the number of recessive sibs, according to equations (32).

On an average, the total weight derived from parents and children in these families is

$$W_{2,M}(s) = 2+sw(1-\nu^4)$$
$$= 2+\frac{4s(1+\nu)(1-\nu)^3}{\{s(1-\nu)(1+3\nu)+3(1+\nu)^2\}}+\frac{s\nu(1-\nu)^2}{s(1-\nu)+(1+\nu)}. \quad (33)$$

When $s=1$, this expression reduces to the maximum likelihood weight, equation (7), so that the scores, though different from those of table 1, are just as good and have the advantage of belonging to a system applicable to any value of s. When $s=2$

$$W_{2,M}(2) = \frac{2(27+2\nu-11\nu^2+9\nu^3-4\nu^4+\nu^5)}{(3-\nu)(5+10\nu-3\nu^2)}, \quad (34)$$

and comparison with equation (9) gives the efficiency shown graphically in fig. 1, always a little higher than Cotterman's. As $s\to\infty$, the

TABLE 3

Weight per sib for most efficient linear scoring of children of two recorded parents

s / p^2	1	2	3	4	5	6	7	8
0·00	1·0000	0·8000	0·6667	0·5714	0·5000	0·4444	0·4000	0·3636
0·05	0·4642	0·3727	0·3120	0·2684	0·2356	0·2100	0·1895	0·1726
0·10	0·3350	0·2719	0·2293	0·1984	0·1749	0·1564	0·1415	0·1292
0·15	0·2583	0·2120	0·1801	0·1567	0·1387	0·1245	0·1130	0·1034
0·20	0·2056	0·1706	0·1460	0·1278	0·1137	0·1024	0·0932	0·0855
0·25	0·1667	0·1398	0·1206	0·1062	0·0949	0·0858	0·0783	0·0720
0·30	0·1367	0·1159	0·1008	0·0893	0·0801	0·0727	0·0666	0·0614
0·35	0·1128	0·0968	0·0848	0·0756	0·0682	0·0621	0·0571	0·0528
0·40	0·0935	0·0811	0·0717	0·0643	0·0583	0·0533	0·0492	0·0456
0·45	0·0775	0·0680	0·0606	0·0547	0·0499	0·0459	0·0424	0·0395
0·50	0·0641	0·0569	0·0512	0·0465	0·0427	0·0394	0·0366	0·0342
0·55	0·0528	0·0474	0·0431	0·0394	0·0364	0·0338	0·0315	0·0296
0·60	0·0432	0·0392	0·0360	0·0332	0·0308	0·0288	0·0270	0·0254
0·65	0·0349	0·0321	0·0297	0·0276	0·0258	0·0243	0·0229	0·0217
0·70	0·0278	0·0258	0·0241	0·0226	0·0213	0·0202	0·0191	0·0182
0·75	0·0215	0·0202	0·0191	0·0181	0·0172	0·0164	0·0156	0·0150
0·80	0·0161	0·0153	0·0146	0·0140	0·0134	0·0128	0·0124	0·0119
0·85	0·0113	0·0109	0·0105	0·0101	0·0098	0·0095	0·0092	0·0089
0·90	0·0071	0·0069	0·0067	0·0066	0·0064	0·0063	0·0061	0·0060
0·95	0·0033	0·0033	0·0032	0·0032	0·0032	0·0031	0·0031	0·0030
1·00	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000

s / p^2	9	10	11	12	13	14	15	16
0·00	0·3333	0·3077	0·2857	0·2667	0·2500	0·2353	0·2222	0·2105
0·05	0·1585	0·1465	0·1362	0·1273	0·1195	0·1125	0·1064	0·1009
0·10	0·1189	0·1101	0·1025	0·0959	0·0901	0·0850	0·0804	0·0763
0·15	0·0953	0·0884	0·0825	0·0773	0·0727	0·0686	0·0650	0·0617
0·20	0·0790	0·0734	0·0685	0·0643	0·0606	0·0572	0·0543	0·0516
0·25	0·0667	0·0621	0·0581	0·0546	0·0514	0·0487	0·0462	0·0439
0·30	0·0570	0·0532	0·0498	0·0469	0·0443	0·0419	0·0398	0·0379
0·35	0·0491	0·0459	0·0431	0·0406	0·0384	0·0364	0·0347	0·0330
0·40	0·0425	0·0399	0·0375	0·0354	0·0335	0·0319	0·0303	0·0290
0·45	0·0370	0·0347	0·0327	0·0310	0·0294	0·0279	0·0266	0·0255
0·50	0·0321	0·0302	0·0286	0·0271	0·0258	0·0245	0·0234	0·0224
0·55	0·0278	0·0263	0·0249	0·0237	0·0226	0·0215	0·0206	0·0198
0·60	0·0240	0·0228	0·0216	0·0206	0·0197	0·0188	0·0181	0·0174
0·65	0·0205	0·0195	0·0186	0·0178	0·0171	0·0164	0·0157	0·0151
0·70	0·0173	0·0166	0·0159	0·0152	0·0146	0·0141	0·0136	0·0131
0·75	0·0143	0·0138	0·0132	0·0128	0·0123	0·0119	0·0115	0·0111
0·80	0·0115	0·0111	0·0107	0·0104	0·0100	0·0097	0·0095	0·0092
0·85	0·0087	0·0084	0·0082	0·0080	0·0078	0·0076	0·0074	0·0072
0·90	0·0059	0·0058	0·0056	0·0055	0·0054	0·0053	0·0052	0·0051
0·95	0·0030	0·0030	0·0029	0·0029	0·0029	0·0029	0·0028	0·0028
1·00	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000	0·0000

These weights are to be used only when at least one parent is T ; when both parents are *tt*, the weight is zero.

weight approaches the same limit as that in equation (10); thus for large families the score tends to full efficiency, and at small values of ν is therefore much better than Cotterman's.

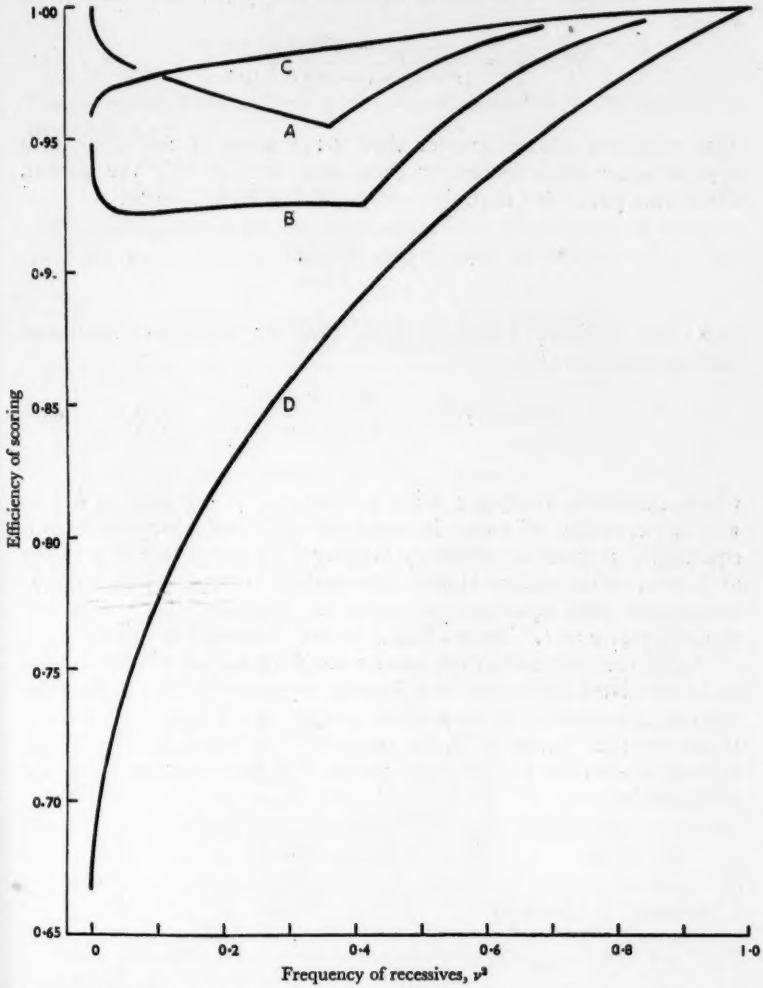

FIG. 1.—Efficiencies of Cotterman's scores and of most efficient linear scores, for families with two recorded parents, type (i).

Curve A: Cotterman, $s = 1$;
Curve B: Cotterman, $s = 2$;
Curve C: Most efficient linear, $s = 2$;
Curve D: Cotterman, $s \to \infty$.

(For $s = 1$ or $s \to \infty$, the most efficient linear score is fully efficient.)

(ii) *One parent recorded*

Cotterman's score for a family record of one parent and s children, the total number of recessives amongst the $(s+1)$ individuals, has weight

$$W_{1,C}(s) = \frac{4(s+1)^2(1+\nu)}{(s^2+3s+4)+\nu(3s^2+9s+4)} \quad . \quad . \quad (35)$$

This weight is always greater than 1 : scoring of the children is always better than discarding them and scoring only the parent. Cotterman points out that, for parent-child pairs, his weight

$$W_{1,C}(1) = \frac{2(1+\nu)}{1+2\nu}$$

shows an efficiency compared with that for maximum likelihood scoring, equation (12),

$$\text{Efficiency} = \frac{8(1+\nu-\nu^2)}{(1+2\nu)(8-7\nu+2\nu^2)}, \quad . \quad . \quad (36)$$

which expression reaches 1 for $\nu = 0$ or $\nu = \frac{1}{2}$, but falls to 8/9 at $\nu = 1$. Insertion of $s = 2$ in equation (35) and comparison with equation (13) gives an efficiency ranging from 72/77 at $\nu = 0$ to 6/7 at $\nu = 1$, with rather higher intermediate values. For large s, comparison with equation (14) shows an efficiency of 4/5 at $\nu = 0$ rising to 1 at $\nu = 1$. These efficiencies are illustrated in fig. 2.

Again the information can be increased by scoring the one parent as an unrelated individual and forming a y-score for the s sibs after separate examination of the possible progeny of a T and of a *tt* parent. If the recorded parent is T, the probability of which is $(1-\nu^2)$, the various possibilities for the other parent and the s children have the probabilities :—

Parents		No. of children		P
Recorded	Unrecorded	T	*tt*	
T	TT	s	0	μ^2
TT	T*t*	s	0	$\frac{2\mu\nu(1-\nu)}{1+\nu}$
T*t*	T*t*	$s-n$	n	$\frac{4\mu\nu^2}{1+\nu}\binom{s}{n}\frac{3^{s-n}}{4^s}$
TT	*tt*	s	0	$\frac{\nu^2(1-\nu)}{1+\nu}$
T*t*	*tt*	$s-n$	n	$\frac{2\nu^2}{1+\nu}\binom{s}{n}2^{-s}$

From these probabilities are found the mean and variance of the number of recessive children

$$\mathrm{E}(n) = \frac{s\nu^2}{1+\nu} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (37)$$

$$\mathrm{V}(n) = \frac{s\nu^2}{4(1+\nu)^2}\{s(1-\nu)(1+3\nu)+(1+\nu)(3-\nu)\} \quad . \quad (38)$$

Similarly, for families having the recorded parent *tt*, the probability of which is ν^2,

$$\mathrm{E}(n) = s\nu, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (39)$$

$$\mathrm{V}(n) = \tfrac{1}{2}\mu\nu s(s+1). \quad . \quad . \quad . \quad . \quad . \quad . \quad (40)$$

Again taking ν_0^2 as a first approximation to ν^2, the number of recessives in either type of family may be used to give an estimate of δ, a first

TABLE 4

Functions required in forming most efficient linear scores for children of one recorded parent

ν^2	$\frac{\nu^2}{2+\nu}$	$\frac{2(1+\nu)^2}{2+\nu}$	2ν
0·00	0·0000	1·0000	0·0000
0·05	0·0050	1·3467	0·4472
0·10	0·0137	1·4959	0·6325
0·15	0·0243	1·6124	0·7746
0·20	0·0365	1·7117	0·8944
0·25	0·0500	1·8000	1·0000
0·30	0·0645	1·8805	1·0954
0·35	0·0799	1·9549	1·1832
0·40	0·0961	2·0247	1·2649
0·45	0·1130	2·0905	1·3416
0·50	0·1306	2·1530	1·4142
0·55	0·1488	2·2127	1·4832
0·60	0·1675	2·2700	1·5492
0·65	0·1867	2·3252	1·6125
0·70	0·2065	2·3784	1·6733
0·75	0·2266	2·4299	1·7320
0·80	0·2472	2·4798	1·7889
0·85	0·2682	2·5284	1·8439
0·90	0·2896	2·5756	1·8974
0·95	0·3113	2·6217	1·9494
1·00	0·3333	2·6667	2·0000

Scores are calculated from s, the total number of sibs, and n, the number of recessive sibs, according to equations (41).

order adjustment to ν_0^2. As before, the procedure may conveniently be put into the form of a score, y, which will lead directly to a revised estimate, ν^2. The scores are :—

$$\left.\begin{aligned}\text{Recorded parent T}: y &= -\frac{s\nu^3}{2+\nu} + \frac{2n(1+\nu)^2}{2+\nu}\\ \text{Recorded parent } tt: y &= -s\nu^2+2n\nu,\end{aligned}\right\} \quad . \quad (41)$$

with a mean weight per sib scored, derived from equations (38), (40), of

$$w = \frac{(1-\nu)^2(2+\nu)^2}{s(1-\nu)(1+3\nu)+(1+\nu)(3-\nu)} + \frac{\nu(1+\nu)}{2(s+1)} \quad . \quad (42)$$

Table 4 (page 381) gives the functions required in scoring with a trial value of ν^2, and w is tabulated in table 5.

The total weight per family, including the parental contribution, is

$$\begin{aligned} W_{1,M}(s) &= 1+sw \\ &= 1+\frac{s(1-\nu)^2(2+\nu)^2}{s(1-\nu)(1+3\nu)+(1+\nu)(3-\nu)}+\frac{s\nu(1+\nu)}{2(s+1)} \quad . \quad (43) \end{aligned}$$

This expression is necessarily at least as great as $W_{1,C}(s)$ for any combination of s and ν. For parent-child pairs ($s = 1$), it reduces to $W_{1,L}(1)$, equation (12), showing the scoring then to be fully efficient. For $s = 2$, comparison with equation (13) shows the scoring to be more than 0·95 efficient (except when ν is very small), and therefore considerably more efficient than the simple count for large ν. As $s \to \infty$, the modified score does not tend to full efficiency, but comparison with equation (14) shows that

$$\text{Efficiency } (y) = \frac{(1+2\nu-2\nu^2)(10-3\nu+\nu^2)}{2(1+3\nu)(5-6\nu+3\nu^2-\nu^3)},$$

an expression which reaches unity at $\nu = 0$, 0·5 and 1, and never falls below 0·994. In large families, the efficiency is thus almost complete, a great improvement on that of the simple count, especially for small ν. These efficiencies are also shown in fig. 2.

(iii) *Neither parent recorded*

For a record relating only to s sibs, no sub-classification can increase the information provided by Cotterman's score ; this is

$$y = n, \quad . \quad . \quad . \quad . \quad . \quad . \quad (44)$$

the number of recessives, and

$$E(y) = s\nu^2. \quad . \quad . \quad . \quad . \quad (45)$$

Cotterman has shown that the weight per sib is

$$w = \frac{4(1+\nu)}{s(1+3\nu)+(3+\nu)} \quad . \quad . \quad . \quad (46)$$

a function which he has tabulated against ν, and which is shown in table 6 tabulated against ν^2. The total weight per sibship is

$$W_{0,C}(s) = \frac{4s(1+\nu)}{s(1+3\nu)+(3+\nu)}. \quad . \quad . \quad . \quad (47)$$

By comparison with equation (16), Cotterman has shown that when $s = 2$ the efficiency rises to unity at about $\nu^2 = 0{\cdot}4$, but that for large or small ν it falls to about 0·9. Though no detailed examination has been made, there is reason to suspect that as s is increased the

TABLE 5

Weight per sib for most efficient linear scoring of children of one recorded parent

s / p^2	1	2	3	4	5	6	7	8
0·00	1·0000	0·8000	0·6667	0·5714	0·5000	0·4444	0·4000	0·3636
0·05	0·7033	0·5430	0·4431	0·3745	0·3244	0·2861	0·2560	0·2316
0·10	0·6197	0·4741	0·3851	0·3247	0·2807	0·2473	0·2211	0·1999
0·15	0·5666	0·4306	0·3487	0·2935	0·2536	0·2233	0·1995	0·1803
0·20	0·5286	0·3991	0·3224	0·2710	0·2339	0·2059	0·1839	0·1662
0·25	0·5000	0·3750	0·3021	0·2536	0·2188	0·1925	0·1719	0·1553
0·30	0·4780	0·3559	0·2858	0·2396	0·2065	0·1816	0·1621	0·1465
0·35	0·4610	0·3406	0·2726	0·2281	0·1964	0·1726	0·1541	0·1392
0·40	0·4480	0·3282	0·2617	0·2185	0·1879	0·1650	0·1472	0·1330
0·45	0·4385	0·3184	0·2527	0·2104	0·1807	0·1586	0·1414	0·1276
0·50	0·4320	0·3107	0·2453	0·2037	0·1747	0·1531	0·1364	0·1231
0·55	0·4282	0·3049	0·2395	0·1982	0·1696	0·1484	0·1321	0·1191
0·60	0·4269	0·3009	0·2349	0·1938	0·1654	0·1445	0·1285	0·1157
0·65	0·4280	0·2986	0·2317	0·1903	0·1620	0·1413	0·1254	0·1128
0·70	0·4314	0·2980	0·2298	0·1879	0·1594	0·1387	0·1229	0·1104
0·75	0·4370	0·2991	0·2292	0·1865	0·1577	0·1369	0·1210	0·1086
0·80	0·4449	0·3020	0·2299	0·1863	0·1569	0·1358	0·1198	0·1073
0·85	0·4551	0·3067	0·2322	0·1873	0·1572	0·1356	0·1193	0·1066
0·90	0·4676	0·3133	0·2361	0·1897	0·1587	0·1365	0·1198	0·1068
0·95	0·4826	0·3221	0·2419	0·1938	0·1617	0·1387	0·1215	0·1081
1·00	0·5000	0·3333	0·2500	0·2000	0·1667	0·1429	0·1250	0·1111

s / p^2	9	10	11	12	13	14	15	16
0·00	0·3333	0·3077	0·2857	0·2667	0·2500	0·2353	0·2222	0·2105
0·05	0·2114	0·1945	0·1801	0·1677	0·1569	0·1474	0·1390	0·1314
0·10	0·1824	0·1677	0·1552	0·1445	0·1351	0·1269	0·1197	0·1132
0·15	0·1645	0·1512	0·1400	0·1303	0·1218	0·1144	0·1079	0·1020
0·20	0·1516	0·1394	0·1290	0·1201	0·1123	0·1055	0·0994	0·0940
0·25	0·1417	0·1302	0·1205	0·1122	0·1049	0·0985	0·0929	0·0878
0·30	0·1336	0·1228	0·1137	0·1058	0·0989	0·0929	0·0876	0·0829
0·35	0·1269	0·1167	0·1080	0·1005	0·0940	0·0883	0·0832	0·0787
0·40	0·1212	0·1114	0·1031	0·0960	0·0897	0·0843	0·0795	0·0752
0·45	0·1164	0·1069	0·0989	0·0921	0·0861	0·0808	0·0762	0·0721
0·50	0·1121	0·1030	0·0953	0·0887	0·0829	0·0779	0·0734	0·0694
0·55	0·1085	0·0996	0·0921	0·0857	0·0801	0·0752	0·0709	0·0671
0·60	0·1053	0·0967	0·0894	0·0831	0·0777	0·0729	0·0687	0·0650
0·65	0·1026	0·0941	0·0870	0·0808	0·0755	0·0709	0·0668	0·0631
0·70	0·1003	0·0920	0·0849	0·0789	0·0737	0·0691	0·0651	0·0615
0·75	0·0985	0·0902	0·0832	0·0773	0·0721	0·0676	0·0636	0·0601
0·80	0·0972	0·0889	0·0819	0·0760	0·0708	0·0664	0·0625	0·0590
0·85	0·0964	0·0880	0·0810	0·0751	0·0699	0·0655	0·0616	0·0581
0·90	0·0964	0·0879	0·0807	0·0747	0·0695	0·0650	0·0611	0·0576
0·95	0·0974	0·0886	0·0813	0·0751	0·0698	0·0652	0·0612	0·0576
1·00	0·1000	0·0909	0·0833	0·0769	0·0714	0·0667	0·0625	0·0588

general level of efficiency of the score at first decreases. Comparison with equation (17), however, shows that for large sibships the efficiency is very high, being unity for $\nu = 0$, 1 and never falling below 0·97. For families of intermediate size the scoring may not be very satis-

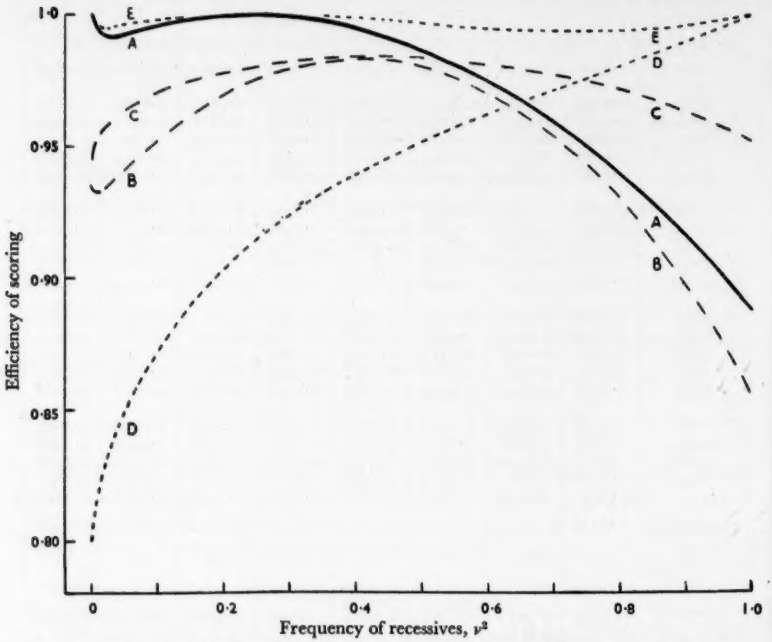

FIG. 2.—Efficiencies of Cotterman's scores and of most efficient linear scores, for families with one recorded parent, type (ii).

Curve A : Cotterman, $s = 1$;
Curve B : Cotterman, $s = 2$;
Curve C : Most efficient linear, $s = 2$;
Curve D : Cotterman, $s \to \infty$;
Curve E : Most efficient linear, $s \to \infty$.
(For $s = 1$, the most efficient linear score is fully efficient.)

factory, but no improvement is likely to be effected without very considerable complications. Efficiencies for $s = 2$ and for large s are shown in fig. 3.

With the aid of the tables that have been provided, the technique of scoring is simple, and may be summarised as follows. A provisional estimate, ν_o^2, of the proportion of recessives in the population is used to give the score and weight per individual for each family recorded. A revised estimate is then obtained by summation over all records, leading to

$$\nu^2 = \frac{\Sigma wy}{\Sigma ws}. \qquad . \quad . \quad . \quad . \quad (48)$$

TABLE 6

Weight per sib for most efficient linear scoring of children of unrecorded parents

σ^2 \ s	2	3	4	5	6	7	8	9
0·00	0·8000	0·6667	0·5714	0·5000	0·4444	0·4000	0·3636	0·3333
0·05	0·7455	0·5943	0·4940	0·4227	0·3694	0·3281	0·2950	0·2680
0·10	0·7299	0·5746	0·4738	0·4031	0·3508	0·3105	0·2785	0·2525
0·15	0·7196	0·5621	0·4611	0·3909	0·3392	0·2996	0·2683	0·2429
0·20	0·7120	0·5528	0·4518	0·3820	0·3308	0·2918	0·2610	0·2361
0·25	0·7059	0·5455	0·4444	0·3750	0·3243	0·2857	0·2553	0·2308
0·30	0·7008	0·5394	0·4384	0·3693	0·3190	0·2808	0·2507	0·2265
0·35	0·6965	0·5343	0·4334	0·3645	0·3145	0·2766	0·2469	0·2229
0·40	0·6927	0·5298	0·4290	0·3604	0·3107	0·2731	0·2435	0·2198
0·45	0·6893	0·5259	0·4251	0·3568	0·3073	0·2699	0·2407	0·2171
0·50	0·6863	0·5224	0·4217	0·3536	0·3044	0·2672	0·2381	0·2147
0·55	0·6836	0·5193	0·4186	0·3507	0·3017	0·2647	0·2358	0·2126
0·60	0·6811	0·5164	0·4158	0·3481	0·2993	0·2625	0·2338	0·2107
0·65	0·6788	0·5138	0·4133	0·3457	0·2971	0·2605	0·2319	0·2090
0·70	0·6767	0·5114	0·4110	0·3435	0·2951	0·2586	0·2302	0·2074
0·75	0·6747	0·5091	0·4088	0·3415	0·2932	0·2569	0·2286	0·2059
0·80	0·6729	0·5071	0·4068	0·3396	0·2915	0·2553	0·2271	0·2046
0·85	0·6712	0·5051	0·4049	0·3379	0·2899	0·2539	0·2258	0·2033
0·90	0·6696	0·5033	0·4032	0·3363	0·2884	0·2525	0·2245	0·2021
0·95	0·6681	0·5016	0·4015	0·3348	0·2870	0·2512	0·2233	0·2010
1·00	0·6667	0·5000	0·4000	0·3333	0·2857	0·2500	0·2222	0·2000

σ^2 \ s	10	11	12	13	14	15	16
0·00	0·3077	0·2857	0·2667	0·2500	0·2353	0·2222	0·2105
0·05	0·2456	0·2266	0·2103	0·1962	0·1839	0·1730	0·1634
0·10	0·2309	0·2127	0·1972	0·1838	0·1721	0·1618	0·1526
0·15	0·2219	0·2043	0·1892	0·1762	0·1649	0·1549	0·1461
0·20	0·2155	0·1982	0·1835	0·1708	0·1598	0·1501	0·1415
0·25	0·2105	0·1935	0·1791	0·1667	0·1558	0·1463	0·1379
0·30	0·2065	0·1898	0·1755	0·1633	0·1527	0·1433	0·1351
0·35	0·2031	0·1866	0·1726	0·1605	0·1500	0·1408	0·1327
0·40	0·2003	0·1839	0·1700	0·1581	0·1477	0·1387	0·1306
0·45	0·1978	0·1816	0·1678	0·1560	0·1458	0·1368	0·1288
0·50	0·1955	0·1795	0·1659	0·1542	0·1440	0·1351	0·1273
0·55	0·1936	0·1776	0·1641	0·1526	0·1425	0·1337	0·1259
0·60	0·1918	0·1760	0·1626	0·1511	0·1411	0·1324	0·1246
0·65	0·1902	0·1745	0·1612	0·1497	0·1398	0·1312	0·1235
0·70	0·1887	0·1731	0·1599	0·1485	0·1387	0·1301	0·1225
0·75	0·1873	0·1718	0·1587	0·1474	0·1376	0·1291	0·1215
0·80	0·1861	0·1706	0·1576	0·1464	0·1366	0·1281	0·1206
0·85	0·1849	0·1695	0·1565	0·1454	0·1357	0·1273	0·1198
0·90	0·1838	0·1685	0·1556	0·1445	0·1349	0·1265	0·1190
0·95	0·1828	0·1676	0·1547	0·1436	0·1341	0·1257	0·1183
1·00	0·1818	0·1667	0·1538	0·1429	0·1333	0·1250	0·1176

Unrelated individuals, including parents of recorded children as explained earlier, are scored as 0 if T, 1 if *tt*, with $w = 1$, $s = 1$ in the formula. It is quite legitimate to score certain sizes of families with maximum likelihood x-scores, where tables for these are available,

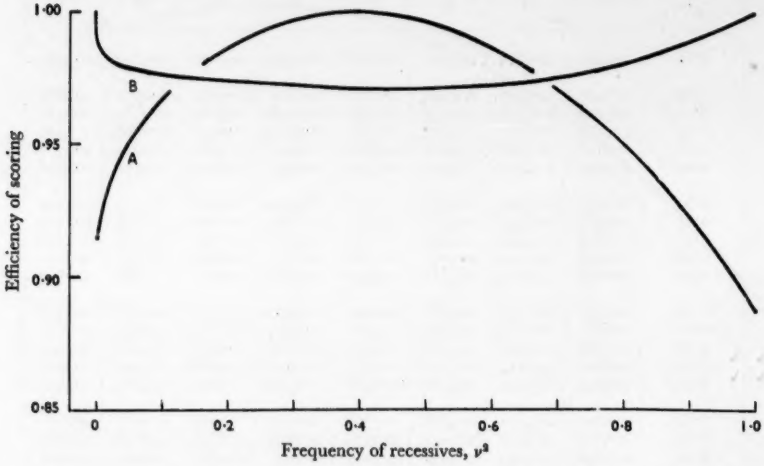

FIG. 3.—Efficiencies of Cotterman's or most efficient linear scores, for families with no recorded parent, type (iii).

Curve A : $s = 2$;
Curve B : $s \to \infty$.

in which case x replaces the corresponding wy in the numerator of equation (47), W_L replaces the corresponding ws in the denominator. If ν^2 differs much from ν_o^2, the whole data should be re-scored using ν^2 as a provisional estimate. When the final estimate has been formed, its variance is

$$\hat{V}(\nu^2) = \frac{\nu^2(1-\nu^2)}{\Sigma ws} \quad . \quad . \quad . \quad . \quad . \quad (49)$$

An example follows in section 4.

4. NUMERICAL EXAMPLE

Boyd and Boyd (1941) record presence or absence of the ability to taste phenyl-thiocarbamide for 58 families of Armenians from Ghazir and Beyrouth (Syria). Of these families, 5 have both parents classified, 14 have one parent, and 39 consist of sibs only (in 3 instances, only one sib being classified). The factor t for taste-blindness is generally considered to be inherited as a simple recessive, and the records of the 58 families are consistent with this hypothesis. On the assumption that the population studied was mating at random

in respect of the taste factor, the frequency of the t gene may be estimated by the methods of section 3. The first stage of the calculations is shown in table 7.

TABLE 7

Estimation of frequency of t *gene from records of 58 Armenian families*

(*a*) Parents

Frequencies of		Sum of values of	
T	*tt*	W	*x*
20	4	24	4

(*b*) Children (scored for $v_0^2 = 0{\cdot}25$)

s	Recorded parents	No. of families	Numbers of children		Sum of values of		
			T	*tt*	*w*	*y*	*s*
2	T, T	1	2	0	0·140	−0·25	2
	T, *tt*	1	2	0	0·140	−1·00	2
3	T, T	1	3	0	0·121	−0·38	3
	T, *tt*	1	2	1	0·121	0·75	3
4	T, T	1	4	0	0·106	−0·50	4
1	T	2	1	1	0·500	1·70	2
	tt	2	1	1	0·500	0·50	2
2	T	7	9	5	0·375	8·30	14
3	T	3	5	4	0·302	6·75	9
1	none	3	1	2	1·000	2·00	3
2	none	26	38	14	0·706	14·00	52
3	none	8	21	3	0·546	3·00	24
4	none	2	7	1	0·444	1·00	8

Part (*a*) of table 7 shows the phenotypes of all recorded parents for families of types (i) or (ii) (sections 2 and 3). In part (*b*), the phenotypes of the children are classified according to parental types and numbers of sibs; the three sibs with no recorded parents and $s = 1$, of course, might have been grouped with the parents as unrelated individuals. Of a total of 152 persons recorded, 36, or 24 per cent., are recessive non-tasters. Scoring with a trial value $v_0^2 = 0{\cdot}25$, therefore seems appropriate. For the parents, the maximum likelihood x-score is simply the total number of recessives, and the weight to be attached to the score is the total number of persons. The most efficient linear scores in part (*b*) of the table are constructed as described in section 3. For example, the y-score for

the family of 3 sibs with parents T, *tt* is taken from the second of equations (32), which, using table 2 for $\nu^2 = 0{\cdot}25$, gives

$$\begin{aligned} y &= -3 \times 0{\cdot}500 + 1 \times 2{\cdot}250 \\ &= 0{\cdot}75. \end{aligned}$$

The weight per sib is read from table 3, in the column for $s = 3$ and the row for $\nu^2 = 0{\cdot}25$, as $w = 0{\cdot}121$. Similarly, the total score for the 7 families of 2 sibs with one T parent and the other parent unrecorded is obtained from the first of equations (41), with the aid of table 4, as

$$\begin{aligned} y &= -14 \times 0{\cdot}050 + 5 \times 1{\cdot}800 \\ &= 8{\cdot}30, \end{aligned}$$

with a weight per sib $w = 0{\cdot}375$ (from table 5). By summation over the whole of part (*b*) of table 7

$$\begin{aligned} \Sigma wy &= 20{\cdot}034, \\ \Sigma ws &= 68{\cdot}046. \end{aligned}$$

Therefore the revised estimate of the population frequency of recessives is

$$\begin{aligned} \nu^2 &= \frac{4 + 20{\cdot}034}{24 + 68{\cdot}046} \\ &= \frac{24{\cdot}034}{92{\cdot}046} \\ &= 0{\cdot}2611, \end{aligned}$$

a result so close to the provisional ν_0^2 that obviously no re-scoring is needed. The variance of the estimate is

$$\begin{aligned} \mathrm{V}(\nu^2) &= \frac{0{\cdot}261 \times 0{\cdot}739}{92{\cdot}046} \\ &= 0{\cdot}00210. \end{aligned}$$

The conclusion is that in the population studied 26·1 per cent. $\pm$ 4·6 per cent. of persons are non-tasters, an estimate which corresponds to a frequency 0·511 for the *t* gene.

5. SUMMARY

Most efficient linear scores have been developed for the estimation of the frequency of recessives, in a population mating at random for a factor involving two allelomorphic genes which show simple dominance. The general theory of estimation by iterative scoring systems has been applied in order to obtain formulæ for these scores, and for families of sibs having two, one, or no recorded parents. The method is more efficient than Cotterman's (1947) estimation from a simple count of recessives, the gain being greater than in the absence of dominance ; it is, of course, in general less efficient than

maximum likelihood estimation, but the latter is scarcely practicable as a routine method except for very small families.

With the aid of tables given in section 3, the method may be used almost as simply and expeditiously as Cotterman's. An example in section 4 shows its application to the estimation of the frequency of phenyl-thiocarbamide taste-blindness from a sample of 58 Armenian families; the estimate formed is that 26·1 per cent.±4·6 per cent. of the population are non-tasters.

REFERENCES

BOYD, W. C., AND BOYD, L. S. 1941.
Data for testing for genetic linkage on 500 pairs of sibs.
Ann. Eugen., Lond. 11, 1-9.

COTTERMAN, C. W. 1947.
A weighting system for the estimation of gene frequencies from family records.
Cont. Lab. Vert. Biol., Univ. Michigan 33.

FINNEY, D. J. 1948.
The estimation of gene frequencies from family records. I. Factors without dominance.
Heredity 2, 199-218.

FISHER, R. A. 1940.
The estimation of the proportion of recessives from tests carried out on a family not wholly unrelated.
Ann. Eugen., Lond. 10, 160-170.

BASAL STERILE MUTANTS IN SPELTOID WHEAT

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INTRODUCTION

AMONG a number of speltoids discovered in a crop of Yeoman II wheat in 1929-30, one plant was sterile in the basal floret of most spikelets. Its descendants throughout the years remained constant. Crossed with Yeoman II it gave an F_2 segregation of 1 Yeoman : 2 basal-fertile heterozygous speltoids : 1 basal-sterile homozygous speltoid. When crossed with an unrelated *vulgare* variety, Victor, the homozygous speltoid fraction in the F_2 varied in its basal fertility from fertile to sterile, with all intermediate degrees of fertility. The intermediate basal sterility is expressed in two different ways : either all the heads of one plant are similar, with intermediate frequencies of basal-sterility ; or the heads may differ, providing chimæra-like combinations of different sterility and fertility types. In later generations selection from this F_2 succeeded in extracting a basal-fertile homozygous speltoid. This paper deals with the inheritance of basal fertility in these speltoids, and by implication in related forms of *Triticum vulgare*.

Two further speltoids with higher but variable sterility, which were recently obtained from A. T. Pugsley, Waite Agricultural Research Institute, Adelaide, enhanced the interest in the determination of fertility relationships in speltoids. These further types have not yet been studied.

DESCRIPTION OF MAIN SPELTOID TYPES

All the parents and F_1s included in this study form 21 bivalents during meiosis. No deletion was detected cytologically (*cf.* Huskins, 1946). This, together with the 1 : 2 : 1 segregation of *vulgare* : heterozygous speltoid : speltoid referred to above, places these speltoids as series α types (*cf.* Huskins, *l.c.*). There was no evidence of structural hybridity in any of the F_1s. Differences in chiasma frequencies between parents and F_1s were of the order to be expected from environmental variation (*cf.* Mather, 1935).

Fig. 1 illustrates diagramatically the normal distribution of sterile and fertile florets in spikelets of the stable speltoid types. There are, however, exceptional spikelets in all types (pl. I).

In selfed progenies of single plants grown in 1934-35, the following frequencies of fertile basal florets were obtained : basal-sterile speltoid 26-28 per cent., basal-fertile speltoid 86 per cent. There is, however, in general a good deal of fluctuation.

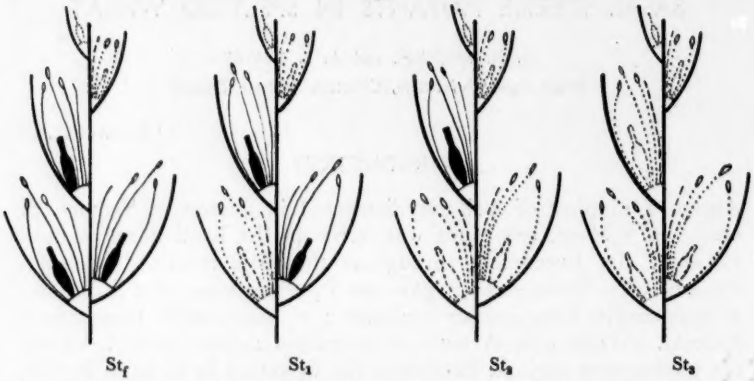

FIG. 1.—Spikelet types in speltoids. Florets drawn in outline are usually sterile but sporadically form grain. Florets drawn in black are normally fully fertile.

Ten ears of the basal-sterile speltoid were dissected shortly before flowering. As in the mature plant, the lemma was fully developed in each floret. The basal floret showed different degrees of development in the following proportions of spikelets :—

Fully developed	15 per cent.
Palea missing	25 „ „
Palea, androecium missing and sometimes gynoecium as well	60 „ „

Observations by Dr I. F. Phipps and Mr A. T. Pugsley generally confirm these sterility relationships in the abortive flowers of their semi-sterile speltoids.

The following symbols are used :—

V—Victor, normal *Triticum vulgare* variety.
Y—Yeoman, normal *Triticum vulgare* variety.
St_1—homozygous speltoid, majority of basal florets sterile.
St_f—homozygous speltoid, majority of basal florets fertile.
St_i—homozygous speltoid, proportion of basal florets sterile.
St_u—homozygous speltoid, chimæra-like combinations of heads with complete basal fertility (as in St_f), complete basal sterility (as in St_1) and partial basal sterility (as in St_i) in the same plant.

RESULTS

The F_2 data presented in the table and fig. 2 permit the following conclusions :—

(i) St_1 and Y have a speltoid fraction in their F_2 consisting only of St_1, and therefore are apparently isogenic for basal sterility (cross 1).

(ii) St_f and V similarly are apparently isogenic for basal fertility (cross 2).

(iii) In crosses between these two groups, the speltoid fraction contains all grades of fertility (crosses 3-5).

(iv) If Y and St_1 were truly isogenic, then the segregations in the speltoid fractions in crosses 4 and 5 should be identical. However, the frequencies in the sterility classes are higher in cross 5 than in cross 4.

(v) Similarly if V and St_f were truly isogenic, the speltoid segregations in crosses 3 and 5 should be identical, which again is not the case.

Results in back and triple crosses confirm those quoted above.

TABLE

Segregation in F_2 of crosses 1-5

(Percentages of fertility types in the speltoid fraction are shown in italics)

Cross no.	Parents	*vulgare*	Het. speltoid	Speltoid				Total
				St_f	St_i	St_u	St_1	
1	$Y \times St_1$	366	814	...	...	...	431 *100·0*	1611
2	$V \times St_f$	192	468	235 *100·0*	...	...	...	895
3	$V \times St_1$	284	561	112 *45·3*	69 *27·9*	30 *14·6*	36 *12·1*	1092
4	$Y \times St_f$	107	278	89 *69·0*	15 *11·6*	19 *14·7*	6 *4·6*	514
5	$St_f \times St_1$	...	...	173 *27·2*	154 *24·2*	234 *36·8*	75 *11·8*	636

DISCUSSION

Since basal sterility is phenotypically correlated with the speltoid character, it seems reasonable to consider linked modes of origin.

According to the theory of the origin of speltoids and the related fatuoids (Winge, 1924 ; Huskins, 1933 ; and recently reviewed by Huskins, 1946), the common feature of series α, β and γ speltoids is the partial or complete deletion of the C chromosome, on which the epistatic genes (S) which determine *vulgare* head characters are located. In the β series the entire chromosome is missing ; the

series γ has an observable deficiency, with every intergradation to the series α speltoids in most of which the deficiency is not cytologically detectable. This deletion allows the expression of hypostatic genes determining speltoid head characters. From the theory of the alloploid origin of hexaploid wheats it is deduced that the speltoid gene or gene complex is located upon the semi-homologous B chromosome (Winge, 1924; Huskins, 1946; McFadden and Sears, 1946). The series β speltoids and fatuoids, which lack the C chromosome altogether, are proof that the speltoid complex is not located on the C chromosome.

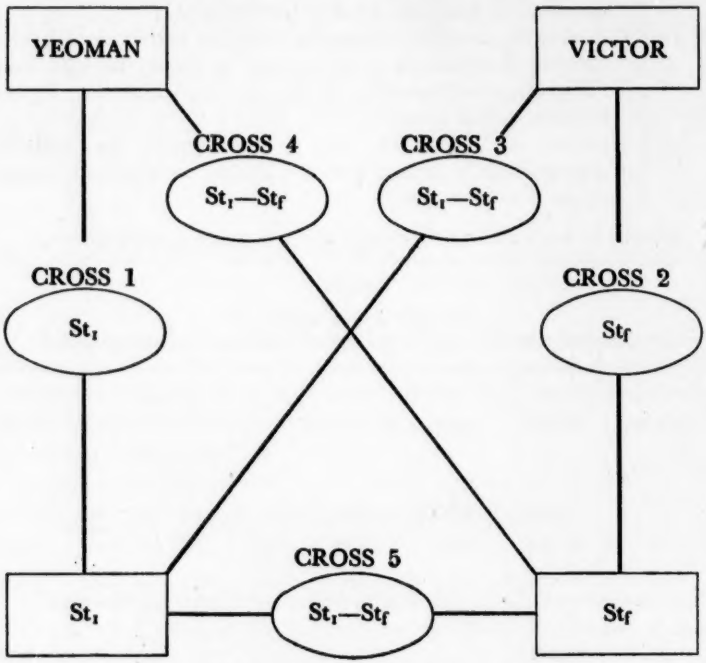

FIG. 2.—Basal fertility in crosses. Parents in rectangles and, in ovoids, fertility grades of the speltoid fractions in F_2.

The observations summarised in (i-v) above present three distinct facts.

(*a*) The difference in basal fertility between *vulgare* and St_1 speltoids. We suggest that this difference is caused by an epistatic gene or gene complex (F) linked to (S) and included in an undetected deletion of the C chromosome responsible for the origin of the speltoid types.

(*b*) The difference in basal fertility between Y and St_1 on one hand, and V and St_f on the other hand. We propose that this be explained

on the basis of gene differences with partial dominance of fertility (G_x/g_x). The location of these genes cannot be defined but it is postulated that one or more (G/g) are located upon the C chromosome external to the deletion.

(*c*) The quantitative differences in basal fertility in the segregating speltoid fractions of the crosses 3-5 (table). We suggest that these are due to differences in the proportions of crossovers and non-crossovers between the breakage point of the deletion (D) and the gene or genes (G/g) which are located upon the C chromosome. On this view the chief difference between crosses 3 and 4, viz. in the frequencies of St_f, is due to the mode of origin of St_f which in cross 3 arises from crossing-over, in cross 4 from segregation. In cross 5, which has two speltoid parents, and hence no crossing-over between D and G/g all fertility classes arise from segregation.

Cross 3.—V by St_1 . . $\frac{SF\ D\ G}{SF\ D\ G} \times \frac{D\ g}{D\ g}$

F_2: 1. *vulgare* types $\frac{SF\ D\ G}{SF\ D\ G}$ $\frac{SF\ D\ G}{SF\ D\ g}$ $\frac{SF\ D\ g}{SF\ D\ g}$

2. Het. speltoid $\frac{SF\ D\ G}{D\ G}$ $\frac{SF\ D\ G}{D\ g}$ $\frac{SF\ D\ g}{D\ g}$

3. Speltoid . $\frac{D\ G}{D\ G}$ $\frac{D\ G}{D\ g}$ $\frac{D\ g}{D\ g}$

Cross 4.—Y by St_f . . $\frac{SF\ D\ g}{SF\ D\ g} \times \frac{D\ G}{D\ G}$

F_2: 3. Speltoid as in cross 3 except that some of the cross-over types are now non-crossovers.

Cross 5.—St_f by St_1 . . $\frac{D\ G}{D\ G} \times \frac{D\ g}{D\ g}$

F_2: 3. Speltoids now all non-crossovers.

FIG. 3.—Segregation for basal sterility in crosses 3, 4 and 5, on the assumptions of one fertility complex (F) in the speltoid deletion and of another complex (G/g), also located on the C chromosome.

The question must be asked as to the nature of what has been referred to as the gene complex for basal fertility (G_x/g_x). Evidence

is available from many simple, back and triple crosses, but the most direct comes from the cross V by St_1, of which a large material was grown up to F_6. In this cross neither of the two parent genotypes was obtained in an F_2 of 107 plants which were tested in F_3. It is therefore likely that the minimum number of required genes is two. No concrete suggestions would be fruitful as to the number of genes actually involved, in view of the possibility of dominance in opposite directions and in view of the great difficulty of diagnosis imposed by fluctuation.

Selection, commenced in F_2, succeeded by F_5 in establishing a line with full fertility, which has since remained constant. Selection for basal sterility was not carried out as consistently and a homozygous line was not obtained in the limited periods during which selection was practised. The proportion of basal sterile plants was, however, rapidly increased.

The theory proposed above postulates that at least one gene for basal fertility (G/g) in addition to the gene or gene complex (F) is situated upon the C chromosome. Where the further gene or genes for fertility are located cannot be defined. The location of another epistatic "normal" gene (F) upon the C chromosome, in addition to those previously recorded (*cf.* Huskins, 1946), further emphasises the importance of this chromosome in the evolution of *Triticum*.

Basal sterility, although rare in the *Hordeæ*, is widespread in the *Gramineæ* as a whole. This in itself argues for its antiquity, and when, as in the basal-sterile speltoids, it is combined with an archaic character, it suggests an origin beyond its present generic confines. Yet the fact that among the many speltoid wheats reported so far only a few with basal sterility have been found, does not support the idea of a widespread occurrence of g_x in *Triticum vulgare*. It remains to be seen whether g_x is common in Yeoman, and whether it occurs in one or both of its parents.

The fact that basal sterility mutants occur raises the question as to the determination of basal fertility in the A and B chromosome sets. Typically both of these condition basal fertility. The mutant reported here tends to suggest that the F gene has taken charge of basal fertility to such an extent as to inactivate corresponding complexes in other sets.

SUMMARY

Sterility of the basal floret in a series *a* speltoid wheat is caused by genes hypostatic to elements included in the speltoid deletion of the C chromosome. It is suggested that at least some of the hypostatic genes are situated on the C chromosome of *Triticum vulgare*.

Acknowledgments.—We are greatly indebted to Dr C. D. Darlington, who read the manuscript, for valuable suggestions and criticisms, and to Mr J. B. Hair for his assistance in classifying most of the speltoid material used in this study.

FIG.

FIG. 1

FIG. 1.—Progressive sterility in speltoids. Top St_1, middle St_2, bottom St_3.

FIG. 2

FIG. 2.—Base-sterile speltoid, type St_1. There ar two base-fertile spikelets near the tip of the ear.

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REFERENCES

HUSKINS, L. 1933.
The origin and significance of fatuoids, speltoids and other aberrant forms of oats and wheat.
Proc. Worlds Grain Exhib. and Conf. 11, 45-49.

HUSKINS, L. 1946.
Fatuoid, speltoid and related mutations of oats and wheat.
Bot. Rev. 12, 457-514.

M^{c}FADDEN, E. S., AND SEARS, E. R. 1946.
The origin of *Triticum Spelta* and its free threshing hexaploid relatives.
J. Hered. 37, 81-89, 107-116.

MATHER, K. 1935.
Chromosome behaviour in a triploid wheat.
Z. f. Zellforsch. 23, 117-138.

WINGE, Ø. 1924.
Zytologische Unterschungen über Speltoide und andere mutantenähnliche Aberranten beim Weizen.
Hereditas 5, 214-286.

Rh FREQUENCY IN THE PEOPLE OF LUCKNOW

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HUMAN erythrocytes contain a variety of antigenic components—A and B, M and N and the Rh series. The agglutinogen Rh has been shown to have no relation with the ABO groups.

The importance of the Rh series lies in the fact that an Rh negative woman who is pregnant with a fœtus whose blood cells are positive can develop an antibody against the agglutinogen, and in addition, any Rh negative person may form such an antibody against the agglutinogen if transfused with Rh positive blood. Rh positive infants usually develop jaundice or anæmia, or are still-born if the mother has developed the Rh antibody. Recent studies in the U.S.A., however, point to the fact that the incompatibility of the Rh factor in mother and fœtus is unimportant in the production of premature abortions, and in the etiology of hæmolytic icterus, ectopic pregnancies, etc. (Schwartz and Levine, 1943). In any case, the genetics of the Rh factor and the understanding of it have increased tremendously in recent years, and the racial significance of its varying frequencies, as well as those of the agglutinogens M and N is now becoming clear. As Mourant (1947) puts it the present Rh ratio in Western Europe is an unstable one, and one is led to postulate a mixing of populations predominantly Rh-positive and Rh-negative respectively within the last few thousand years.

Khanolkar and Sanghvi, in discussing their results, suggest that " differences between our (their) results and those of other workers in India may be due to the fact that they have been using serum (after suitable dilutions) of animals immunised with red cells of rhesus monkeys." They say further, that " such immunisation leads to the development of anti-Rh as well as other agglutinogens to a variable extent."

During a racial and serological survey of Bengal, which I carried out in 1945 at the invitation of Prof. P. C. Mahalanobis, financed by the Indian Statistical Institute, Calcutta, I was supplied with some dried human anti-Rh testing sera from the Galton Serum Unit at Cambridge, sent by Dr G. L. Taylor for use during the Survey. When the Bengal Survey was completed in July 1945, I had to return to duty at Lucknow, and I brought some of the sera with me for testing people there. Without further absorption, the anti-Rh sera

from England could not be used against any group other than A and O, so that these alone were tested. The technique followed was that of Taylor (1943).

TABLE 1

Tests against A and O

Subjects	A		O		Total	
	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−
BRAHMINS :						
Male . . .	8	0	5	0		
Female . . .	6	1	3	0	22	1
KAYASTHAS :						
Male . . .	5	0	9	0		
Female . . .	6	0	2	0	22	0
MUSLIMS :						
Male . . .	9	1	6	0		
Female children .	5	0	6	0	26	1
CHAMARS :						
Male . . .	4	0	6	2		
Female . . .	6	0	5	0	21	2
DOMS :						
Male . . .	5	1	7	0		
Female . . .	3	0	4	1	21	2
Total . .	57	3	53	3	112	6
Percentage of Rh− 5·45						

TABLE 2

Frequencies of Rh− individuals in various populations

Population	Number examined	Percentage	Authority
Chinese	132	1·5	Wiener, Sonn and Belkin (1945)
Indians	100	2·0	Khanolkar and Sanghvi (1945)
Lucknow Indians . .	116	5·45	Majumdar (see table 1)
Negroes (U.S.A.) . .	223	8·1	Wiener (1944)
Jews (Canada) . . .	967	8·37	Lubinski, Benjamin and Strean (1945)
Calcutta Indians . .	200	10·0	Greval and Chowdhury (1943)
,, ,, . .	240	10·0	Das Gupta (1944)
American and English (white)	...	15·0	Landsteiner and Wiener (1940)
South Americans . .	112	17·86	Invernizzi and Yannicelli (1944)
Basques (Argentine) . .	250	35·6	Etcheverry (quoted by Mourant (1947))

The results of the Bengal Survey have been published in full (Majumdar and Kishen, 1947).

SUMMARY

The present results agree with the general scheme of world variation in the Rh gene which we may arrange as in table 2. Here

we see that the Indian populations stand between the low extreme in China and the high extreme in the Basque population.

REFERENCES

DAS GUPTA, C. R. 1944.
Ind. med. Gaz. 37, 372.

GREVAL, S. D. S., AND CHOWDHURY, A. B. R. 1943.
J. Ind. med. Ass. 13, 65.

INVERNIZZI, D., AND YANNICELLI, S. 1944.
Arch. Uruguayo med. Cir. : y Esp. 25 (2), 185.

KHANOLKAR, V. R., AND SANGHVI, L. D. 1945.
Nature, Lond. 155, 427-428.

LANDSTEINER, K., AND WIENER, A. S. 1940.
Proc. Soc. exp. Biol. N.Y. 42, 223.

LUBINSKI, H. B., BENJAMIN, —., AND STREAN, G. J. 1945.
Canada med. Ass. J. 53, 28.

MAJUMDAR, D. N., AND KISHEN, K. 1947.
Eastern Anthropologist 1, 1.

MOURANT, A. E. 1947.
Nature, Lond. 160, 505.

SCHWARTZ, H. H., AND LEVINE, P. 1943.
Amer. J. Obs. Gyn. 46, 827.

TAYLOR, G. L. 1943.
Proc. Soc. Med. 36, 225.

WIENER, A. S., SONN, E. B., AND BELKIN, R. B. 1944.
Amer. J. Phys. Anth. N.S., *2*, 187.

WIENER, A. S., SONN, E. B., AND BELKIN, R. B. 1945.
J. Immunol. 50, 6.

GENETICAL SOCIETY OF GREAT BRITAIN

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THE RESPONSE TO SELECTION FOR SIZE IN *DROSOPHILA*

F. W. ROBERTSON and E. C. R. REEVE

Four strains from a wild stock of *Drosophila melanogaster* are being selected for long and short wing, long and short thorax. A closed pedigree system avoids intense inbreeding. Divergence of long and short thorax strains has been slow. After 40 generations the long wing strain had increased 6 per cent. in wing and thorax; the short wing strain had decreased 24 per cent. in wing length and 13 per cent. in thorax length. Rate of divergence has been fairly steady for 30 generations.

Inbred back-selection lines from long-wing strain reverted quickly to unselected level, then changed little; those from short wing strain remained stationary after a small initial advance. A mass-mated line without selection from long-wing strain returned to unselected level, while a similar line from short-wing strain remained unchanged.

Long and short-wing strains show a striking sex-linked difference. The difference in wing size between these two strains reflects mainly a difference in cell size.

Crosses between long-wing and long-thorax strains show heterosis.

SELECTION FOR SIZE IN MICE

D. S. FALCONER

An experiment on selection for size in mice is in progress. Crosses were first made between two pairs of inbred lines, and then the two F_1s were crossed to produce a large F_2 generation. From this two selection lines were started, one for large size and one for small size, the criterion of selection being weight at 6 weeks. A comparison of the variance in the first two generations gave the data necessary for the estimation of the degree of heritability of the measurement, and from this estimate of heritability the expected divergence of the two lines after the first selection was calculated. The estimate of heritability was based on two assumptions; that genetic variance was absent from the inbred lines and therefore from the F_1 generation; and that non-additive gene action (dominance and epistasis) was negligible. A comparison of the predicted divergence of the two selected lines with that actually obtained will indicate the extent to which these assumptions were justified.

HERITABILITY OF ALL-OR-NONE CHARACTERS IN POULTRY

A. ROBERTSON and I. M. LERNER

A simple method for the estimation of the degree of heritability of all-or-none characters has been evolved. It is here applied to the case of mortality in poultry, with special reference to the production flock of the University of California at

Berkeley, California. The data permit a partition of the mortality into its separate causes such as lymphomatosis, reproductive disorders, etc., and allow a consideration to be made of the method of inheritance of resistance to death from different causes. In addition, it is possible to relate the heritability of mortality with its level of incidence.

THE DEGREE OF GENETIC ISOLATION BETWEEN VARIOUS LOCAL POPULATIONS OF DAIRY CATTLE

A. ROBERTSON and A. ASKER

The cline in milk production in England and Wales may be due to genetic or management reasons. An attempt is here made to estimate the genetic diversity of animals in different regions of the country by considering the migration of sires and dams in the Friesian and Shorthorn breeds. It is found that the sires move much more than dams and that the general rate of migration is such as to render the chance of formation of distinct sub-populations fairly small. Both breeds have fairly definite areas which act as a centre for the production of breeding stock.

POTENTIAL IMPROVEMENT IN MILK YIELD WITHIN BREEDS OF DAIRY COWS

J. M. RENDEL

Calculation based on several estimates of the heritability of milk yield in a closed herd of dairy cows shows that improvement from straightforward selection of dams and sires is of the order of 1 to 2 per cent. per year. Progeny testing would reduce the yearly improvement. Comparison of the performance of the progeny of bulls, which have daughters yielding in two types of herd, with their dams and with the means of the herds in which they produce, suggests that much of the difference between recorded herds at the present day is environmental.

THE SEX RATIO IN SOME HUMAN CONGENITAL ABNORMALITIES

B. WOOLF

In collections of pedigrees of human genetic abnormalities it is quite exceptional to find a normal sex ratio among the affected individuals. Excess of affected males is more common than excess of affected females. Infant deaths and stillbirths attributed to congenital malformations show a similar picture, the male/female ratio ranging from about 4 for pyloric stenosis to 0·4 for anencephalus. For most abnormalities, no explanation of the disturbed sex ratio has been advanced. In several of the cases in which partial sex linkage has been postulated, it will be shown that this hypothesis is not in accord with all the facts, and that a more likely explanation is that the condition is more lethal for the female than for the male fœtus. This theory was advanced by Hogben to account for a male excess among albinos, and was opposed by Haldane by an argument which will be shown to contain flaws in both the algebra and the arithmetic. Differential sex lethality will be shown to be a plausible explanation in a number of other abnormalities, including Sjögren's pedigrees of oligophrenia. Physiological reasons will be given, supplemented by data from vital statistics, for anticipating a sex difference in the lethality of abnormal conditions among mammals. Since excess lethality seems to act most often against the female, doubt is cast on the belief that there is an excess of males at fertilisation, and this proposition will be shown to rest on statistical fallacies.

DOUBLE MOSAICS IN THE PROGENY OF CHEMICALLY TREATED *DROSOPHILA*

C. AUERBACH

The term " double mosaics " is used to describe individuals which are mosaic for two mutations at the same locus, or for two re-arrangements involving the same loci. Several such were found in the progeny of mustard gas treated *Drosophila* males. Their significance for the theory of mosaicism after chemical treatment will be discussed.

INDUCTION OF GIANT CELLS IN AMPHIBIA BY ENZYME POISONS

R. A. BEATTY

Chloracetophenone (C.A.P.), a tear-gas, is an inhibitor of enzymes whose activity depends on —SH groups, and is also an inhibitor of glycolysis and respiration.

Rana temporaria eggs at the gastrula or earlier stages were subjected to C.A.P. in minute concentrations ($2-10\times10^{-6}$M). The substance was highly toxic, especially to early stages, and differentially attacked the vegetal pole. At certain critical concentrations tadpoles of a characteristically spotted appearance were produced, due to the presence of giant cells derived from the pigmented epidermal cells, the unpigmented cells being unaffected. Nucleus and cytoplasm of giant cells were on the average three or four times the volume of normal cells. The nuclei appeared on indirect grounds (size, number of nucleoli) to be polyploid, but chromosome counts have not yet been possible. Nuclear division without cell division occurred, polynucleate cells resulting. In spite of their size, these cells were normally differentiated epidermal cells forming part of the epidermal layer, their nucleo-cytoplasmic ratio was normal, and in one case a mitosis at late telophase was observed in a giant cell and in nearby normal cells, suggesting that it had reacted normally to some local mitotic stimulus. Work is proceeding on reversal of the effect of C.A.P. by supplying substances containing —SH groups.

The effects parallel closely those known to be caused by hexenolactone, believed also to be concerned in suppression of enzymes whose action depends on —SH groups.

PARTIAL DEFICIENCIES IN *DROSOPHILA*

B. M. SLIZYNSKI

Drosophila melanogaster eggs treated with 500 r units at 12-14 hours after laying develop into larvæ in which salivary gland chromosomes show structural changes occurring only in parts of the bundles of chromonemata of which salivary chromosomes consist. All known types of changes have been observed, including even those which usually cannot pass through cell division. The yield of changes is very high.

A NEW STRAIN OF LUXATE MICE

T. C. CARTER

In 1945 a silvered mouse, bred by a fancier, was used to introduce silvering into one of the lines being set up by Professor Fisher at the Department of Genetics, Cambridge University ; and in 1946 mice with preaxial polydactyly of the hindfeet occurred in the F_2 generation. The F_3 included mice which resembled Rabaud's " souris luxées," and were accordingly called " luxate." The fancier has since reported having found similar mice in his stocks.

It has since been established that luxate mice are homozygous for a single mutant gene, *lx*. The homozygous expression is variable. Its minimal form consists of triphalangy of the hallux, with or without a prehallux. Its maximal form includes loss of preaxial hind digits, loss of tibia, reduction of femur and pubis, distortion of ischum and ilium, reduction of lumbar vertebræ : groups of muscles are reduced : hydronephrosis is common.

The heterozygote is either normal (60 per cent.) or has a prehallux on one or both hindfeet : the hallux is normal.

Fertility is normal in heterozygotes, but not all homozygotes can breed.

lx is closely linked to macrocytic anæmia, W^v.

The abnormality is recognisable in twelve-day embryos by a proliferation of the preaxial margin of the hindfoot plate.

FACTORS AFFECTING THE FEMALE REPRODUCTIVE SYSTEM IN *DROSOPHILA MELANOGASTER*

R. A. BEATTY

In wild-type adults, growth of the ovary and oocyte were studied to provide a set of data for comparison of mutants. Ovary and oocyte grow rapidly for three or four days after eclosion ; thereafter the ovary increases slightly in length for at least a fortnight. Stocks on a yeasted medium eclose with the ovary about a day in advance of those on ordinary medium. Impregnation had little effect on ovary and oocyte growth. Erratic fecundity was not due to a failure of ovary growth. Mean egg length differed in two strains.

Females of twelve female-sterile stocks were examined. A variety of pleiotropic effects was observed, some of the more striking being parovaria absent and oocytes abnormal (oc ptg^3) ; parovaria rudimentary (ap^4) ; ventral receptaculum rudimentary and eggs small (b rn px sp) ; two or more follicles fused end to end (cg) ; ovaries of mid-pupal size and structure but with asynchronous proliferation of the oogonia (nw^3).

Impregnation of mutant and wild-type females is thought to be conditional on the ovary reaching a certain critical size (some 0·60 mm. in length).

Fecundity of all twelve mutants was less than in wild-type.

Comparison of the mutants indicates that the female internal genitalia can develop independently of one another, with the exception that parovaria do not seem to develop unless spermathecæ are present.

A PHYSIOLOGICAL STUDY OF ISOLATED NUCLEI

H. G. CALLAN

The ultra-structure of the nuclear membrane of isolated germinal vesicle nuclei from amphibian oocytes has been studied by means of the polarising and electron microscopes. The nuclear membrane consists of protein and is completely devoid of lipoid materials. It has a thickness of the order 150 Ångström units. It is an elastic structure capable of stretching to a limit of one and a half times its original linear dimensions while preserving perfect elasticity, and within this range shows a linear increase of birefringence with tension. Above the elastic limit no increase of birefringence can be observed.

Photographs taken with the electron microscope show that the nuclear membrane consists of two component structures. One is a non-fibrous membrane with a granulation close to the limit of resolution of the microscope. Adhering to this membrane is a porous sheet of somewhat thicker material, the pores being about 100 Ångström units in diameter arranged largely in " hexagonal close packing." Experimentally the two membrane components may be detached from one another.

The nuclear membrane has been found permeable to water, salts, sugars and the tetranucleotides of ribose and desoxyribose nucleic acids. Although the problem of permeability has not yet been tackled systematically, it is evident that rate of penetration is roughly universely proportional to molecular size. Fat soluble substances do not show preferential speeds of penetration, in keeping with the absence of lipoids in the membrane.

Egg Albumen, Glycogen, Acacia and synthetic water-soluble celluloses are unable to penetrate, and these substances may be used in the preparation of physiological solutions for the maintenance of isolated nuclei where they counteract the colloid osmotic pressure of the nuclear sap. This osmotic pressure is of the order 30 cm. of water.

The *p*H of the nuclear sap has been studied with special indicators and lies between 6 and 7. It is not markedly different from that of the cytoplasm. A study of the metabolic activity of the nucleus has been initiated. At physiological *p*H and at 25° C. the isolated nucleus from a fully grown frog oocyte consumes about 0·009 mm.^{3} O_2 per hour, while the intact oocyte consumes 0·200 mm.^{3} The nucleus occupies about one sixty-fourth of the volume of an entire oocyte and thus its respiratory activity is by no means negligible, as some authors have recently maintained.

THE NUTRITIONAL REQUIREMENTS OF *DROSOPHILA MELANOGASTER*

C. M. BEGG

The need for a chemically defined medium on which *Drosophila* would grow normally under conditions of aseptic culture arose primarily from work involving environment-sensitive mutants. The provision of such a medium is an indispensable preliminary to investigations into the biochemistry of gene action. A medium containing casein, dextrose, tryptophane, ergosterol, cholesterol, aneurin, riboflavine, nicotinic acid, pyridoxin, biotin, inositol, pantothenic acid, choline, para-aminobenzoic acid, folic acid, ribose nucleic acid and salts allows pupation in from 8 to 14 days from oviposition. Two further fractions seem to be involved before completely normal growth is attained—and results based on such a medium cannot be held to be valid unless growth is shown to be completely normal. One of these factors can be extracted from yeast with hot sodium bicarbonate, and precipated with hydrochloric acid and alcohol. The other is insoluble in all solvents so far tried. There is also some evidence that thymine is involved. Normal growth is also obtained if whole yeast autolysate is added. At the time of writing, suggestive results are being obtained with (*a*) hæmoglobin and (*b*) strepogenin preparations, but it is too early to be definite.

A suitable technique for the sterile culture of *Drosophila* has been worked out.

STUDIES ON THE ARISTOPEDIA ALLELOMORPHS

C. H. WADDINGTON and R. FREEDMAN

The gene ss^a converts the antenna of *Drosophila* into a leg-like organ, and also reduces the size of the tarsus. The two apparently opposed actions are enhanced similarly by temperature treatment of the larvæ, both being increased by cold. Within any one stock, the correlation between leg effect and antennal effect is fairly high ; but the correlation is different in different allelomorphs, some of which affect the antenna proportionately more and some the legs. The effect of temperature is directly on the animal and not a secondary effect of changes in the flora in the culture vessel. The results cannot easily be reconciled with the hypothesis that there are quantitative differences between the allelomorphs.

PRODUCTION OF PHENOCOPIES IN *DROSOPHILA* BY CHEMICAL SUBSTANCES

J. M. MACDONALD

Rapoport reports the production of phenocopies by chemical substances. Six of the substances used by him have been tested by us in live yeast cultures and have been found to give effects similar to those described by him. Thus Boron (Sodium Metaborate), Mercury (Mercuric Chloride), Silver (Silver Nitrate), Thiocyanate ion (Potassium Thiocyanate), Semicarbazide ion (Semicarbazide Hydrochloride), and Fluorine (Sodium Fluoride) give respectively phenocopies of " Eyeless or Aristoapedia," " Minute," " Yellow or Straw," " Chubby," " Black " and " Benign." The Boron-produced phenocopies appear to resemble more closely " Deformed " or " Lobed " than " Eyeless," and in addition " Antennaless " flies are also obtained. The Silver brought about a bleaching of the setæ and body colour generally rather than a true " Yellow " or " Straw " effect. In addition the pericardial cells were coloured reddish brown.

The same substances were tested in sterile (dead yeast) cultures. In all cases the lethal dose was very much lower than in live yeast. Mercury, Boron and Silver gave phenocopies at concentrations which were just sub-lethal, while Fluorine, Thiocyanate ion and Semicarbazide ion produced only normal flies.

The time of action of Boron lies within the first forty-eight hours of larval life. Aristapedia flies (ss^a40a-) are much less sensitive to Boron both as regards the morphogenetic and lethal effect than are Or S flies.

Flies treated with all the above poisons, taken together, show a slight increase in the lethal mutation rate over untreated controls.

HISTOCHEMICAL STUDIES IN EARLY EMBRYOGENESIS OF *DROSOPHILA MELANOGASTER*

YAO TSUNG

Fixed —SH groups are present in the oocyte in all stages. Distribution is diffuse except in some concentration in the cortical ooplasm. In the developing embryo the ectoderm and its derivates show more fixed —SH groups than the other tissue, but it is not clear that any morphogenetical role is played by such substances. In early cleavage stages they are present in the mitotic chromosomes.

Alkaline phosphatase cannot be demonstrated histochemically in the ovary or testis, but acid phosphatase has a wide distribution in both organs. Alkaline phosphatase appears suddenly in the embryo at about the eighth or ninth hour after laying (at 25° C.). The enzyme, which is mostly of intra-nuclear origin, appears first in the ventro-lateral ectoderm of the thoracic region and spreads from there in all directions but disappears from most tissues (except salivary gland and a few others) before hatching. Acid phosphatase is present throughout the embryonic stages, located mostly in nuclei and yolk.

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